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MULTIPLE PRIMARY MALIGNANT NEOPLASMS*†

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THIS study of the occurrence of more than one primary malignant newgrowth afflicting the same person is based on the records of 2,124 patients with malignant tumors treated at The Mayo Clinic in the calendar year 1929. Only cases in which there was a proved microscopic diagnosis of at least one malignant newgrowth while the patient was at the clinic in the course of 1929, were included in the 2,124.

This study was undertaken primarily to determine the relationship of the grade of malignancy to the frequency of occurrence of multiple primary malignant neoplasms. Broders' system of grading malignant neoplasms was used. The age, sex and family history of the patients were included.

The literature on multiple primary malignant tumors has grown to be extensive since Billroth reported the first case in 1869. Hanlon, working at The Mayo Clinic, reviewed the postulates established by Billroth for determining multiple primary malignancy and showed that they are impossible to fulfill in a large percentage of cases of true and undisputed primary multiple malignant neoplasms.

The criteria of Goetze for diagnosis of multiple primary malignant neoplasms, as given by Hanlon, are reasonable and will be followed in this study. They are: (1) the tumors must have the macroscopic and microscopic appearance of the usual tumors of the organs involved; (2) exclusion of metastasis must be certain, and (3) diagnosis may be confirmed by the character of the individual metastasis. Hanlon thoroughly reviewed the literature and reported 48 cases. He concluded that double primary carcinomas are probably incidental.

The work of some investigators, which Hanlon did not have occasion to mention in the published abstract of his thesis is relevant here. Orr expressed the belief that accurate record of the occurrence of multiple malignant neoplasms may decide the disputed question as to whether one

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malignant neoplasm exercises an inhibitory effect on the development of a second. His figures are insufficient to settle that question, but they tend to show that the incidence of multiple primary malignant neoplasms is about what would be expected, if the occurrence were accidental.

Seecof, in commenting on the incidence of multiple primary malignant growths, was inclined to believe, with Ewing, that the "rather common occurrence of two or more tumors in different or the same organs of the same subject, suggests nothing more than an accidental coincidence, in several organs, of the general biological factors in the genesis of tumors." But Ewing also suggested that "when minute search is carefully conducted and all forms of tumor growth included, the proportion of multiple tumors is much greater." It will be noted that these quotations from Ewing are concerning tumorous growths in general.

Williams in conclusion of a chapter on multiple primary carcinoma wrote: "Multiple outbreaks of malignant disease are probably less exceptional than is generally believed, the evidence to this effect being especially strong in the case of paired organs," and "taken in their entirety, these various considerations point to some general systemic change, as the predisposing cause of primary multiplicity."

Woolley's expressed views in this respect are: "Whether the active proliferation is initiated by one or another factor, this would seem evident; that the factor to be effective, the cells must be in a special state, so that whatever acts upon one organ, may affect similarly its pair."

Fried stated that two or more malignant growths occurring at the same time are rare, being found only once in a thousand postmortem examinations. He advanced two reasons for this rarity, and for the average greater age of persons dying with two or more primary malignant neoplasms. (1) A state of immunity is conferred by the first malignant tumor. This belief is based on the experimental work of Jensen and Ehrlich which tended to show that a transplanted malignant growth in mice, confers immunity against a second inoculation of a malignant growth. Later workers, however, have shown that this immunity diminishes after a period. (2) A person with a malignant growth possibly succumbs to his disease before another malignant neoplasm develops. Many workers hold that neoplasms of senescent persons are less malignant than those of younger persons, and it is obvious that persons with mildly malignant neoplasms will live longer and have more chance of a second malignant tumor developing. The latter reason possibly accounts for the fact that most observers believe that persons with multiple primary malignant neoplasms are of more advanced age than persons having only one malignant newgrowth.

Müller found 1,121 subjects with carcinomas at 5,012 postmortem examinations of subjects who were more than twenty years of age. Of these 1,121 subjects, 18 had more than one carcinoma.

The literature on this subject, and the reports of cases, are confusing because there is no agreement as to classifications of malignant growths in general, or as to what constitutes a condition of multiple primary malignant neoplasm.

Billroth's postulates, which were used as criteria by some of the earlier writers, would exclude a large percentage of the type of cases which have been used by later workers. Most of the statistics found in reports in the literature are based on material obtained at necropsy, and it is evident that such reports usually will include only those cases in which there were two or more primary malignant tumors at the time of death. Studies based on a series of postmortem examinations would not take into account the fact that the person dying of one tumor might have been cured of a previous separate and distinct primary malignant newgrowth. It is also conceivable that many small lesions, such as early epitheliomas of the skin, or some small growth in the organs usually not examined postmortem, would escape attention when the internal organs were the center of the examiner's interest.

Few of the reports in the literature include cases in which there were malignant tumors in paired organs or in the same organ, because of the difficulty of excluding the possibility that the second lesion was metastatic. It is known that cysts of the ovary are often bilateral, and it is also known that certain types of these cysts often become malignant, so it would seem that in cases of bilateral papillary carcinoma of the ovaries the evidence is in favor of two or more primary growths rather than of metastasis from one ovarian tumor to the other ovary. It is agreed by most authorities that polyps of the intestinal canal are often the forerunners of malignant tumors. In cases of multiple polyps, in which more than one malignant growth is found, the conclusion would be that two separate malignant growths had developed on two separate polyps. It is hard

to conceive of multiple basal cell epitheliomas, or widely separated squamous cell epitheliomas of low grade being produced from a single focus. The question concerning simultaneous carcinoma in both breasts, or of carcinoma in one breast, subsequent to that in the other, is not so easily settled. Harrington, in a series of 3,038 cases of carcinoma of the breast, found twenty in which the condition was bilateral. He considered the growths to have been primary in each breast. In 42 of the remaining cases, carcinoma developed in the opposite breast subsequently. In most cases carcinoma of the second breast is considered primary by Harrington, except in cases in which the lesion is obviously metastatic.

Kilgore, in studying 1,100 unselected cases of carcinoma of the breast found the second breast, in 37 instances, to be the site of carcinoma, either simultaneously or subsequently. In 13 of these cases, he considered the lesions were definitely primary, and basing his estimate on statistics of the Census Bureau, he concluded that a woman who has had one breast removed for carcinoma, and survives five years after operation, is about four times more likely to have carcinoma in the remaining breast than is the average woman of her age to have carcinoma of either of her breasts.

Owen, who was cited by Hanlon, has reviewed a large number of cases of malignancy seen at the Barnard Free Skin and Cancer Hospital; multiple malignant tumors were found in 4.7 per cent of 3,000 cases. Although the present study is somewhat similar to Owen's, the material used is different. His cases were from a clientele which presents a relatively large percentage of cutaneous carcinomas, and those on which this report is based were from a clientele which presents a large number of patients with carcinoma of the internal organs. Owen also used some cases in which only a clinical diagnosis was made.

PRESENT STUDY

As has been said, there were 2,124 patients at The Mayo Clinic in 1929, in whose cases a microscopic diagnosis of malignant neoplasm was made. At the time of this study, 71 of these patients had also had another primary malignant neoplasm or more. This shows the percentage of proved cases of multiple malignant neoplasm in this series to have been 3.34. These cases were reviewed in 1931, or two years after one of the malignant tumors in each case had been observed. There were 15 patients whose first malignant tumor existed more than two years before 1929; that is, in 1926 or before. Therefore, it is reasonable to assume that about 15 of the 2,053 patients with single malignant tumors seen in 1929 will, at some time later than 1931, have another primary malignant tumor. This reasoning would bring the total number of persons with multiple primary malignant tumors, past and future, to a theoretic total of 86, or 4 per cent of the 2,124 cases of malignant neoplasms reviewed.

If this study included, also, those cases in which only clinical diagnosis was made, the percentages of cases of multiple primary malignant neoplasms would have approached or exceeded those of Owen.

There were 152 separate malignant lesions among the 71 patients. In making this count and subsequent counts patients who were reported to have multiple lesions, but in whose cases the number of such lesions was not stated, are counted as having only 2 lesions. Of the 152 lesions, 58 originated in the skin, and were divided as follows: 37 squamous cell epitheliomas (Figs. 1 and 2), 11 basal cell epitheliomas, 9 mixed basal cell and squamous cell epitheliomas and one melano-epithelioma. There were 31 malignant neoplasms of the breasts, 18 of the ovaries, 17 of the colon, 3 of the parotid glands, and 1 each of the stomach, gall bladder, thyroid gland, and testis, all of which were adenocarcinomas. Of the 10 neoplasms of the uterus, 8 were adenocarcinomas (Figs. 3 and 4), 1 a squamous cell epithelioma,

and 1 a sarcoma. There were 4 squamous cell epitheliomas of the mouth, pharynx and larynx, and 4 of the urinary bladder (Figs. 5 and 6) and urethra. Metastatic squamous cell epitheliomas of the lymph nodes were found in 2 cases in which the

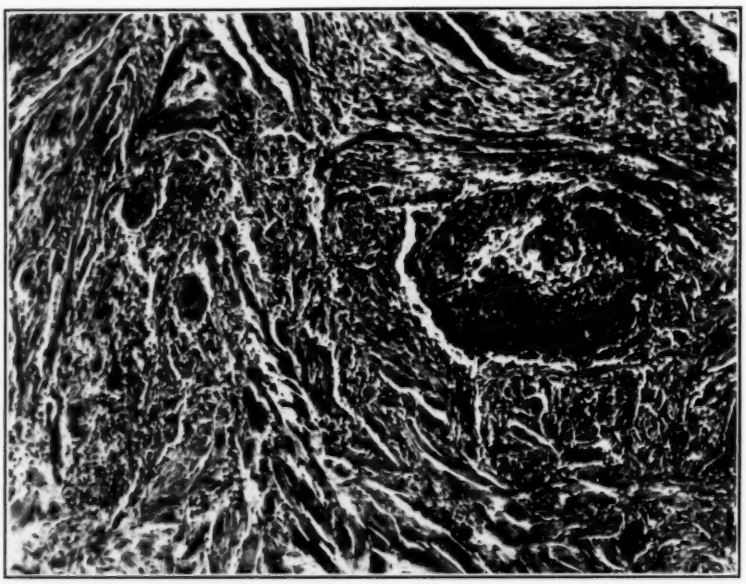

Fig. 1.—(Case 8). Squamous cell epithelioma of the upper jaw, Graded 2 (×110).

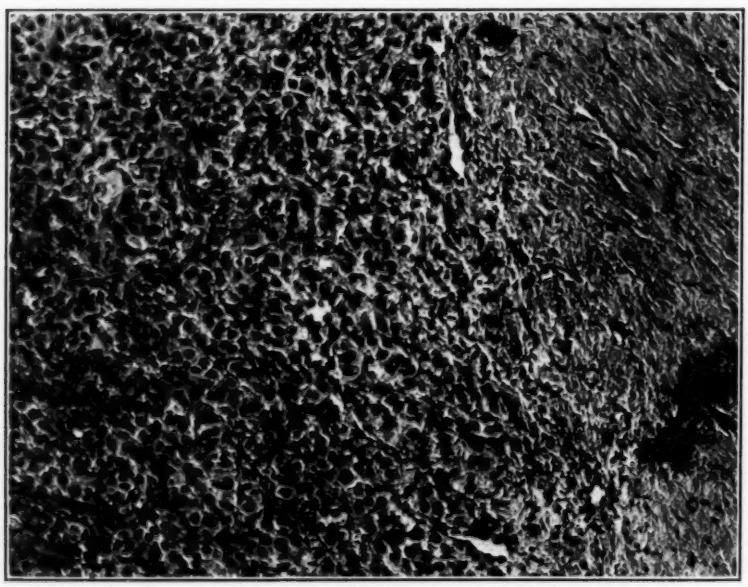

Fig. 2.—(Case 8). Squamous cell epithelioma of the lower lip, Graded 4 (×110).

primary lesion was not known. There was one case of lymphosarcoma of the inguinal region.

Table I includes data on each of the 71 cases.

For better study, the cases have been divided into seven groups. Some cases of necessity are included in more than one group.

Group 1. Squamous Cell Epithelioma of the Skin.—Thirty-two patients had

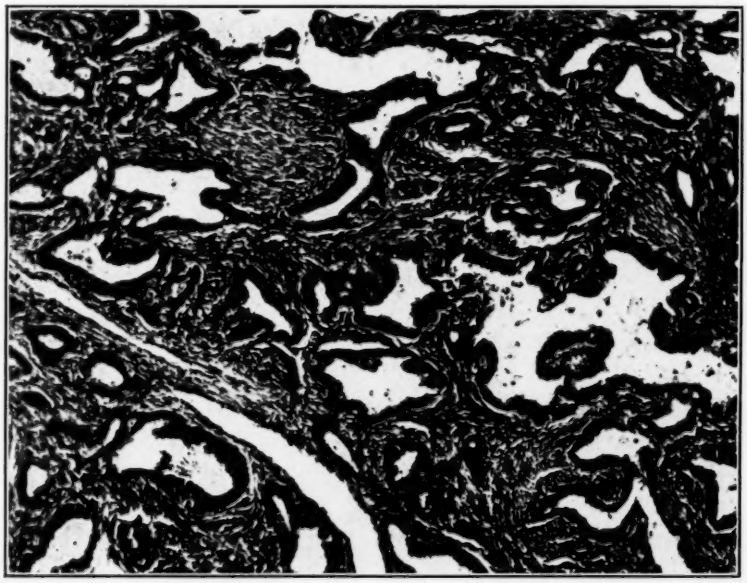

Fig. 3.—(Case 61). Adenocarcinoma of the body of the uterus, Graded 1 (×75).

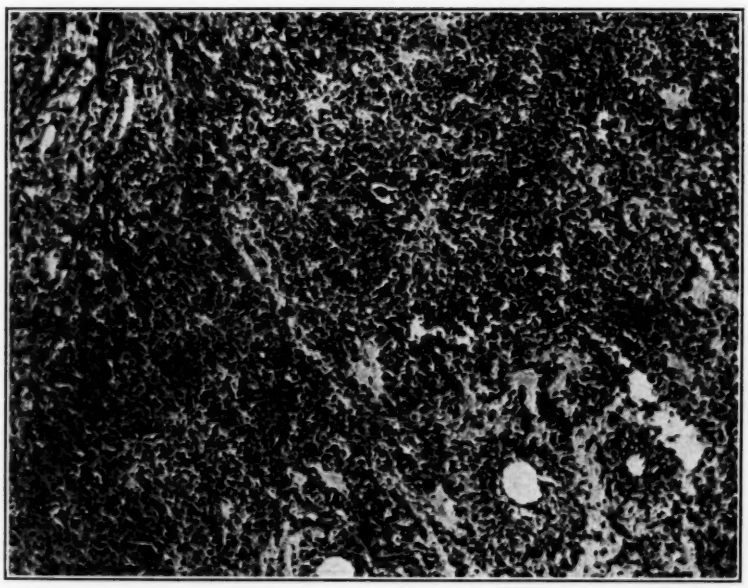

Fig. 4.—(Case 61). Adenocarcinoma of the body of the uterus, Graded 4 (×75).

one or more squamous cell epitheliomas of the skin. In these 32 cases, there were 46 squamous cell epitheliomas of the skin, 9 of which were superimposed on basal cell epithelioma. There were in this group 27 men and 5 women, 28.1 per cent of whom

gave family histories of malignant neoplasms. The average* age at which these lesions appeared was fifty-seven and five-tenths years. The average* grade of malignancy for the group was 1.8.

Fig. 5.—(Case 70). Squamous cell epithelioma of the urinary bladder, Graded 1 (×110).

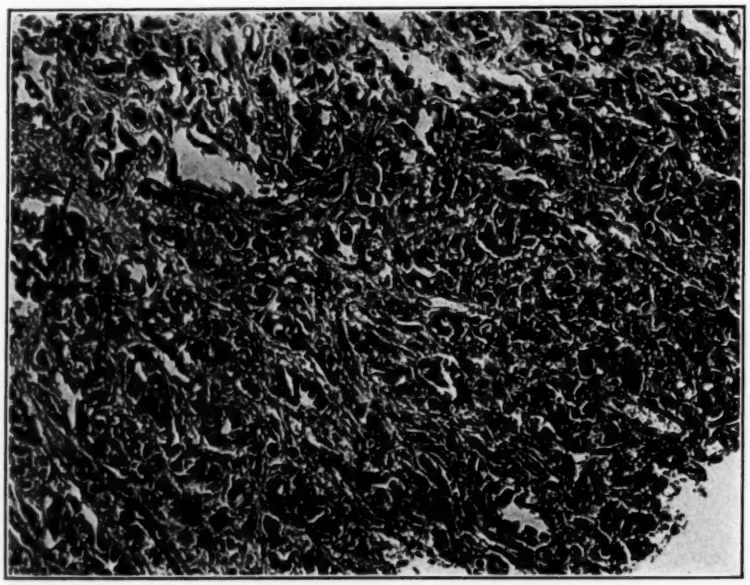

Fig. 6.—(Case 70). Squamous cell epithelioma of the urinary bladder, Graded 4 (×110).

*As far as our material is concerned, the manner of computing the average in this group and elsewhere in the paper, unless the term "average" is qualified, can best be explained by an example: Say that there were two patients in a group. The first growth of one patient was found when he was fifty-two years of age; the second growth when he was sixty. The first growth of the other patient was found when he was sixty years of age; the second growth when he was sixty-eight. The average age for this group would be the average of all the ages just given, or sixty. Say that one of these two patients had two growths graded, respectively, 1 and 3. The other patient had two growths graded respectively, 2 and 4. The average grade of malignancy for the group, therefore, is 2.5.

Group 2. Basal Cell Epithelioma.—In this group there were sixteen patients, of whom twelve were men and four were women. Of the group 37.5 per cent gave family histories of malignant neoplasm. The average age at which the basal cell epitheliomas appeared was fifty-three and four-tenths years.

Group 3. Neoplasms of the Large Bowel.—This group comprises 9 patients, 5 men and 4 women. Six of these patients had more than one primary malignant neoplasm of the large bowel, and 5 had malignant neoplasms other than of the bowel. The age given, in most of the cases, is the age of the patient at the onset of symptoms. The average age when symptoms were noticed was forty-four and three-tenths years. There was a family history of malignant neoplasms in 44.4 per cent of the cases.

Group 4. Neoplasms of the Breast.—Carcinoma of the breast afflicted 18 persons. More than one primary malignant neoplasm of the tissue of the breast occurred in 13, one of whom had two different carcinomas in the same breast (Case 39). The lesions of the breast appeared at an average age of forty-seven and five-tenths years. One patient with carcinoma of the breast was a man (Case 31).

The grades of malignancy of the 31 newgrowths in the breast were as follows: Grade 1, six growths (19.4 per cent); Grade 2, two growths (6.4 per cent); Grade 3, eleven growths (35.5 per cent); and Grade 4, twelve growths (38.8 per cent). The average grade of malignancy was 2.9.

Of the 18 persons who had carcinoma of one breast, 12 had carcinoma of the opposite breast; but of the 12 who had bilateral carcinoma of the breast only 3 had demonstrable nodal metastasis. Five years elapsed in one of the patients in whom there was metastasis before the appearance of the growth in the second breast. This leaves only two of the bilateral cases of carcinoma of the breast in which there is any doubt as to whether both tumors were primary.

In Case 47 there was a family history of marked susceptibility to malignant tumors. There was a positive family history in 33.3 per cent of the cases.

Group 5. Neoplasms of the Ovaries.—There were 7 cases of bilateral malignant tumors of the ovary among the total of 11 women who had malignant tumors of ovarian origin. The average grade of malignancy was 2.3, and in 10 per cent of the cases in which the family history was recorded, other members of the family had malignant tumors.

Group 6. Neoplasms of the Uterus.—In 7 of the cases of malignant newgrowths of the female genitalia, the newgrowths were in the uterus, all of which were carcinomas except 2. Of these 2, one was a sarcoma of the uterus, and the other, a squamous cell epithelioma of the cervix. One patient had 2 primary malignant tumors in the uterus (Case 9). The average grade of uterine malignant growths was 2.2. Two patients (28.6 per cent) gave histories of malignant neoplasms afflicting other members of their families. In every case in which glandular epithelium of the uterus was involved, the associated lesion was in the breast or genitalia.

Group 7.—This contains the 3 cases not included in any of the other groups. The tabulated data for this group are shown in Table I, Cases 69, 70, and 71.

COMMENT

Groups 4, 5, and 6.—Attention has been called to the fact that if a malignant neoplasm develops in one organ of a system, another primary malignant neoplasm

TABLE I

SUMMARY OF CASES OF MULTIPLE PRIMARY MALIGNANT NEOPLASM

CASE	SEX	OTHER MEMBERS OF FAMILY WHO HAVE MALIGNANT GROWTHS	FIRST GROWTH			ASSOCIATED GROWTH OR GROWTHS		
			SITUATION AND TYPE OF GROWTH	GRADE OF MALIGNANCY	AGE OF PATIENT	SITUATION AND TYPE OF GROWTH	GRADE OF MALIGNANCY	AGE OF PATIENT
1	M	0	Mouth; squamous-cell epithelioma	1	52	Mouth; squamous-cell epithelioma	3	52
2	F	1	Nose; squamous-cell epithelioma	1	53	Right upper eyelid; squamous-cell epithelioma	4	56
3*	M	0	Left first finger; squamous-cell epithelioma	1	55	Right second finger; squamous-cell epithelioma	2	55
4*	M	0	Finger; squamous-cell epithelioma	1	52	Finger; squamous-cell epithelioma	1	52
5	M	0	Right side of face; squamous-cell epithelioma	2	70	Left side of face; squamous-cell epithelioma	2	70
6	F	1	Left side of neck; squamous-cell epithelioma	2	65	Left outer canthus; squamous-cell epithelioma	2	69
7	M	1	Right cheek; squamous-cell epithelioma	2	52	Left side of lower lip; squamous-cell epithelioma	3	54
8	M	0	Upper jaw; squamous-cell epithelioma	2	60	Lower lip; squamous-cell epithelioma	4	60
9	M	0	Face; basal and squamous-cell epithelioma	1	75	Face; basal and squamous-cell epithelioma	1	75
10	M	0	Face; squamous-cell epithelioma	1	46	Face; basal and squamous-cell epithelioma	1	46
11	F	1	Face; basal and squamous-cell epithelioma	1	30	Nose; basal and squamous-cell epithelioma	2	33
12	M	0	Right inner canthus; basal-cell epithelioma	1	25	Left cheek; squamous-cell epithelioma	1	55
13	M	1	Lower lip; squamous-cell epithelioma	2	75	Left cheek; squamous-cell epithelioma	3	76
						Upper lip; basal and squamous-cell epithelioma	1	76
14	F	3	Left side of face; basal and squamous-cell epithelioma	1	20	Lip; basal-cell epithelioma	1	43
15	M	0	Nose; basal and squamous-cell epithelioma	2	59	Prepuce; squamous-cell epithelioma	1	62
16	M	0	Left preauricular region; basal-cell epithelioma	1	61	Lower lip; squamous-cell epithelioma	2	65
17	M	0	Left cheek; basal-cell epithelioma	1	67	Lower lip; squamous-cell epithelioma	2	77
18	M	1	Peri-anal region; squamous-cell epithelioma	2	64	Back; basal-cell epithelioma	1	64
19	M	0	Lower lip; squamous-cell epithelioma	4	43	Left cheek; basal-cell epithelioma	1	43
20	M	0	Upper lip; basal and squamous-cell epithelioma	1	48	Right parotid gland; adenocarcinoma	2	51
21	F	0	Right parotid gland; adenocarcinoma	1	32	Right heel; squamous-cell epithelioma	1	36

*Workers with roentgen rays.
**Inoperable carcinoma of stomach at age of forty-two years.
†Also an adenocarcinomatous polyp in uterus.

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**Inoperable carcinoma of stomach at age of forty-two years.
†Also an adenocarcinomatous polyp in uterus.

TABLE I (CONTINUED)

CASE	SEX	OTHER MEMBERS OF FAMILY WHO HAVE MALIGNANT GROWTHS	FIRST GROWTH			ASSOCIATED GROWTH OR GROWTHS		
			SITUATION AND TYPE OF GROWTH	GRADE OF MALIGNANCY	AGE OF PATIENT	SITUATION AND TYPE OF GROWTH	GRADE OF MALIGNANCY	AGE OF PATIENT
22	M	0	Lower lip; squamous-cell epithelioma	1	33	Left testis; adenocarcinoma	4	33
23	M	0	Lower lip; squamous-cell epithelioma	1	67	Nasopharynx; squamous-cell epithelioma	4	68
24	M	0	Inside cheek; squamous-cell epithelioma	2	45	Lower lip; squamous-cell epithelioma	1	46
25	M	0	Mouth; squamous-cell epithelioma	1	63	Gland inguinal region; squamous-cell epithelioma	4	63
26	M	1	Lower lip; squamous-cell epithelioma	1	58	Rectum; adenocarcinoma	2	58
27	M	0	Transverse colon; adenocarcinoma	2	51	Lower lip; squamous-cell epithelioma	1	65
			Transverse colon; adenocarcinoma	3	64			
28	M	0	Larynx; squamous-cell epithelioma	2	63	Nose; basal and squamous-cell epithelioma	1	69
29	M	0	Right parotid gland; adenocarcinoma	1	46	Lower lip; squamous-cell epithelioma	2	55
30	M	0	Glands of neck; squamous-cell epithelioma	3	54	Lower lip; squamous-cell epithelioma	2	63
						Nose; squamous-cell epithelioma	2	65
						Inner cheek; squamous-cell epithelioma	2	68
31	M	1	Lower lip; squamous-cell epithelioma	2	68	Breast; adenocarcinoma	3	84
32	M	0	Stomach; adenocarcinoma	3	61	Nose; squamous-cell epithelioma	4	66
33	M	1	Face; basal-cell epithelioma	1	?	Face; multiple lesions; basal-cell epithelioma	1	?
34	F	2	Nose; basal-cell epithelioma	1	33	Right breast; adenocarcinoma	3	52
						Left breast; adenocarcinoma	4	62
35	F	0	Left cheek; basal-cell epithelioma	1	60	Right breast; adenocarcinoma	4	63
36	F	0	Left breast; adenocarcinoma	4	34	Scar left breast; melanoepithelioma	3	38
37	F	0	Uterus; adenocarcinoma	2	59	Right breast; adenocarcinoma	4	61
38	F	0	Left breast; adenocarcinoma	3	40	Ovary; adenocarcinoma	2	47
39	F	0	Left breast; adenocarcinoma	4	43	Same breast; adenocarcinoma	1	43
40	F	1	Left breast; adenocarcinoma	2	42	Right breast; adenocarcinoma	1	43
41	F	0	Left breast; adenocarcinoma	3	51	Right breast; adenocarcinoma	3	52
42	F	0	Right breast; adenocarcinoma	3	36	Left breast; adenocarcinoma	3	37
43	F	0	Left breast; adenocarcinoma	1	38	Right breast; adenocarcinoma	1	39
44	F	0	Left breast; adenocarcinoma	1	41	Right breast; adenocarcinoma	1	42

TABLE I (CONTINUED)

CASE	SEX	OTHER MEMBERS OF FAMILY WHO HAVE MALIGNANT GROWTHS	FIRST GROWTH			ASSOCIATED GROWTH OR GROWTHS		
			SITUATION AND TYPE OF GROWTH	GRADE OF MALIGNANCY	AGE OF PATIENT	SITUATION AND TYPE OF GROWTH	GRADE OF MALIGNANCY	AGE OF PATIENT
45	F	1	Left breast; adenocarcinoma	4	42	Right breast; adenocarcinoma	4	44
46	F	0	Left breast; adenocarcinoma	4	56	Right breast; adenocarcinoma	4	62
47**	F	4	Right breast; adenocarcinoma	3	34	Left breast; adenocarcinoma	3	35
48	F	0	Right breast; adenocarcinoma	3	45	Left breast; adenocarcinoma	2	50
49	F	0	Left breast; adenocarcinoma	4	55	Right breast; adenocarcinoma	4	56
50	F	0	Scar right breast; adenocarcinoma	3	44	Left breast; adenocarcinoma	4	46
51	F	No data	One ovary; papillary adenocarcinoma	2	39	Other ovary; papillary adenocarcinoma	2	39
52	F	0	One ovary; adenocarcinoma	3	66	Other ovary; adenocarcinoma	3	66
53	F	0	One ovary; adenocarcinoma	1	33	Other ovary; adenocarcinoma	2	33
54	F	0	One ovary; adenocarcinoma	4	52	Other ovary; adenocarcinoma	3	52
55	F	0	One ovary; papillary adenocarcinoma	2	35	Other ovary; papillary adenocarcinoma	2	35
56	F	1	One ovary; papillary adenocarcinoma	1	44	Other ovary; papillary adenocarcinoma	1	44
57	F	0	One ovary; adenocarcinoma	3	44	Other ovary; adenocarcinoma	3	44
58	F	0	One ovary; adenocarcinoma	4	60	Uterus; papillary adenocarcinoma	1	60
59†	F	0	One ovary; adenocarcinoma	2	46	Uterus; papillary adenocarcinoma	2	46
60	F	0	One ovary; adenocarcinoma	4	51	Uterus; adenocarcinoma	2	51
						Uterine polyp; adenocarcinoma	1	51
61	F	1	Uterus; adenocarcinoma	4	38	Uterine polyp; adenocarcinoma	1	38
62	F	1	Rectum; adenocarcinoma	2	47	Uterus; squamous-cell epithelioma	2	48
63	F	0	Colon, 3 growths; adenocarcinoma	1 and 2	37 to 45	Uterus; sarcoma	4	45
64	M		Rectum; adenocarcinoma	2	50	Multiple rectal polyp; adenocarcinoma	?	50
65	F	0	Rectosigmoid; adenocarcinoma	3	50	Rectal polyp; adenocarcinoma	3	50
66	F	1	Rectosigmoid; adenocarcinoma	2	24	Rectal polyp; adenocarcinoma	1	24
67	M	0	Rectum; adenocarcinoma	1	42	Rectum; adenocarcinoma	2	42
68	M	0	Rectum; adenocarcinoma	2	53	Urethra; squamous-cell epithelioma	4	54
69	F	0	Urinary bladder; squamous-cell epithelioma	1	68	Gall bladder; adenocarcinoma	3	69
70	M	0	Urinary bladder; squamous-cell epithelioma	1	58	Urinary bladder; squamous-cell epithelioma	4	59
71	F	0	Thyroid; adenocarcinoma	2	29	Left groin; lymphosarcoma	2	32

is more likely to develop in that organ or in an organ of the same system. In the 29 patients with malignant neoplasms derived from the glandular epithelium of the organs peculiar to the female sex, there were only three proved malignant neoplasms in organs that are common to both sexes. A patient who had had carcinoma of both breasts was found to have an inoperable carcinoma of the stomach at operation, but this patient is not included, because no tissue for microscopic diagnosis of the lesion in the stomach was obtained (Case 47).

The Grade of Malignancy.—The relationship of the occurrence of multiple malignant tumors to the degree of malignancy of these tumors is one of the objects of this study.

Since one of us (Broders) in 1920, published the method of grading the malignancy of squamous cell epithelioma of the lip, it has been extended and accepted in the grading of other types of malignant neoplasms in various situations.

The grade of malignancy has been determined for the 152 lesions, and is recorded in Table I. The average grade of malignancy varies directly with the number of lesions occurring in any one age group, as is shown in Table II. In other

TABLE II

GRADE OF MALIGNANCY OF THE NEOPLASMS

AGE GROUPS, YEARS	NEOPLASMS					
	TOTAL NUMBER	NUMBER IN DIFFERENT GRADES				AVERAGE GRADE
		1	2	3	4	
20 to 29	6	4	2			1.33
30 to 39	28	11	8	6	3	2.0
40 to 49	34	14	7	7	6	2.14
50 to 59	37	10	12	6	9	2.48
60 to 69	36	11	11	5	9	2.33
70 and above	11	6	3	2	0	1.64
Total	152	56	43	26	27	2.15

words, if a person has one malignant tumor, the probability of the development of another malignant tumor varies directly with the grade of malignancy of the first tumor, presuming that the person survives long enough for another malignant newgrowth to develop. This leads to the hypothesis that the unknown factors which cause development of a single malignant neoplasm are exaggerated in cases of multiple malignant neoplasm, and express themselves in both the grade of malignancy and the frequency of multiple malignant neoplasms.

If this is true, then the occurrence of multiple primary malignant tumors should be more common than would be expected if they were merely accidental. This study, and that of Owen, tend to substantiate the hypothesis that multiple primary malignant tumors actually are of more than accidental occurrence in spite of the fact that the probability of the development of a malignant tumor is not known for any control group. Also, if the hypothesis is true, then the average grade of malignancy of multiple primary malignant tumors should be higher than in a like group of single malignant tumors. The proof of this is beyond the scope of this study.

Hereditary Factors.—There are many opinions concerning the part that hereditary influences may play in the causation of malignant neoplasms of human beings. The family histories in most clinical records are lacking in detail and thoroughness, and are wholly inadequate for any statistical data of great value.

In the present study, there was some record of the family history in 70 cases. In 20 cases (28.6 per cent) there was a history of malignant tumors in other members of the family. Three patients gave histories of more than one member of the family having malignant tumor.

Case 47 is interesting in this respect. The woman had a carcinoma in one breast, and then in the other at the ages, respectively, of thirty-four and thirty-five years. At the age of forty-two years, at abdominal exploration, an inoperable lesion which was taken to be malignant was found in the stomach. A note on her history said that she came of a "cancer family," and this is substantiated by the fact that her mother and three of her sisters had had carcinoma of the breast.

Sex.—In this series, nothing of interest was found with relation to distribution by sex. Thirty-two of the patients were men and thirty-nine women.

Age.—Hanlon found the average age of patients with multiple carcinomas, as reported in the literature, to be fifty-eight and eight-tenths years. In his own series of cases in which necropsy was performed, the average age was sixty-two and six-tenths years. Among Owen's cases in which ages were given, the average was sixty-two and one-tenth years. Owen, however, did not take into consideration the age at which the lesions appeared.

In the present study, the average age at which the lesions appeared was fifty and four-tenths years. Difficulty was experienced in some cases in estimating the age at which the lesions appeared, and in these cases, as a rule, the age at the onset of symptoms was taken to be the age at which the tumor developed. This is, obviously, an inaccurate method, but the best that could be used.

SUMMARY AND CONCLUSIONS

Persons are more likely to have more than one primary malignant neoplasm than a review of the literature would lead one to believe.

Multiple primary malignant neoplasms occur most commonly in the same organ, or in organs of the same system.

Of the patients with malignant neoplasms seen at The Mayo Clinic in one year, 3.4 per cent have had more than one primary malignant neoplasm. Reasons have been presented to show that the percentage is even higher than this.

From the study of cases of multiple malignant neoplasms, it seems that the factors which cause the development of the tumors also express themselves in the grade of malignancy of the tumors.

A large percentage of patients with multiple primary malignant neoplasms give family histories which include other members who have had malignant tumors.

No conclusion as to the occurrence of multiple malignant neoplasms, with reference to sex, could be drawn.

In this series, the average age at which the growths developed was fifty and four-tenths years.

Seventy-one cases of multiple primary malignant neoplasms have been added to the literature on this subject.

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STUDIES OF EXPERIMENTAL MUSCLE DEGENERATION*

IV. Factors of Carbohydrate Metabolism in Muscle Repair

D. K. Fishback, Ph.D., and H. R. Fishback, Sc.D., M.D., Chicago, Ill.

IN A previous article,[1] we have directed attention to the variations from normal of certain chemical factors in the metabolism of abnormal muscles. In that study a reproducible type of injury of rabbit muscle, with a standard method of trauma, was used.[2] The acute molecular degeneration of muscle resulting was studied in its florid state.

Briefly, the method of injury consisted in contusing the muscle while the animal was under light anesthesia. With the leg extended on a well padded wooden block the gastrocnemius muscle was struck a number of scattered light blows with a light, rubber-covered iron rod. In this manner the injury produced is diffuse, and the depth of trauma can be gauged very well at its inception. The gastrocnemius is well enough isolated that it can be removed easily for study.

A series of animals with such muscle injury was carried through the stage of repair. Complete study was made of the gross and microscopic changes occurring in the muscle during the process of repair.[2]

Associated with this morphologic study, chemical analyses of the muscles were performed for the entire repair series, with a view to correlating the course of carbohydrate metabolism in the stage of development of the injury, with that found during the break-up and removal of the injured muscle and its replacement by new growing muscle cells.

Methods and Results.—The methods used have been presented in the above-

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given references. Results in series were obtained for glycogen, lactic acid, and organic and inorganic phosphorus. These are presented in the accompanying figure.

DISCUSSION

Studies of glycogen distribution in tissues are often made by staining methods with the purpose of obtaining relative proportions of the glycogen present. Glycogen in the tissues, however, is quite labile. Such factors as the length of time elapsing from the death of the animal, or removal of the tissue, to the time the tissue is fixed, the size of the tissue block to be fixed, the amount of handling or trauma of the tissue, and many other things may vary the amount of glycogen present. It is evident that the study of glycogen by the staining method cannot compare in accuracy or informative value with quantitative chemical recovery of glycogen.

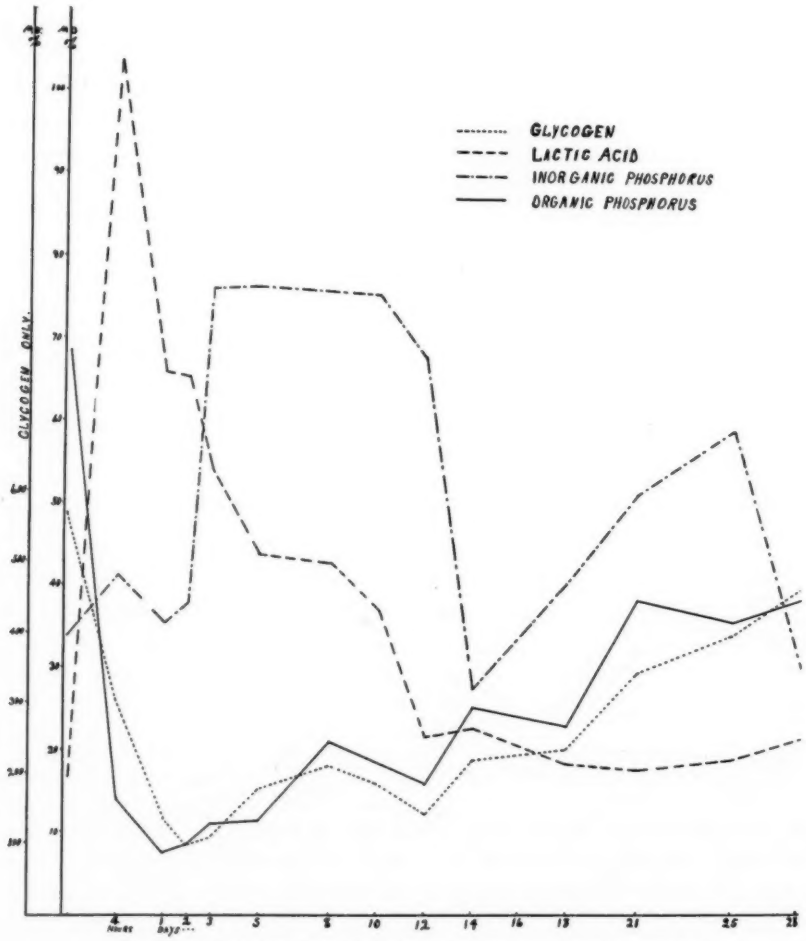

Fig. 1.—Carbohydrate metabolism during muscle repair.

One phase of this question was studied by Naka[3] on brain tissue. It was found that glycogen in stained preparations became visible as granules at a concentration

of about 0.01 per cent when the magnification was 500 times, and that a concentration of about 0.03 per cent was necessary to see granules at a magnification of 50.

In the given figure glycogen decreases quite rapidly to a low point of 94 mg. per cent at the end of forty-eight hours. From that point there is a gradual rise to nearly the control value at the end of the observation period. From microscopic study of repair of the muscles[2] the glycogen increase in repair is found to be synchronous with the new growth of muscle cells.

Craciun[4] found that the glycogen content of regenerating muscle was lower than normal but varied considerably in different parts of the tissue. His conclusions are based on staining of glycogen in histologic sections, which, as previously stated, is open to criticism in regard to either absolute or relative values.

It might be expected that rapidly growing muscle cells would contain much glycogen for use in growth metabolism. In embryonic muscle tissue glycogen has been stated to appear early, and to accumulate in rich amounts.[5, 6, 7] However, it would appear from our studies that in repair of this type of injury in muscles, there is no marked storage of glycogen as a reserve supply. It is formed, instead, in amounts somewhat proportionate to those found in normal muscle.

Lactic acid shows a very rapid rise to a high point of 103.3 mg. per cent in four hours, and a rather rapid decrease thereafter. Its formation might be expected to run parallel to the decrease of glycogen but, as is seen, the lowest glycogen is reached only after forty-eight hours.

The early accumulation of lactic acid, then, is not accounted for by the amount of glycogen used up. A similar discrepancy, under different experimental conditions was noted by Olmsted and Coulthard[8] in their work on fatigued muscles, and by Ronzoni[9] in muscle hash. It is possible that the early excess lactic acid might arise from lactacidogen if sufficient quantities of that substance were present.[10] It is evident that it cannot have had origin from hexose phosphate because of the relatively insignificant increase of inorganic phosphorus at the time of highest lactic acid value.

The later fall of lactic acid, while glycogen continues to be broken up, may be accounted for by the high diffusibility of lactic acid, so that it is rapidly taken up by the circulating blood. It is not likely that the degenerated muscle cells function at a higher level to oxidize increased amounts of lactic acid. As repair progresses lactic acid is seen to decrease gradually down to almost control value.

The phosphocreatine curve runs somewhat parallel to that of glycogen, with a sharp drop during the twenty-four and forty-eight hour periods, and a later gradual rise during the period of repair, although the final value of both is considerably under that of the control. There is, however, no direct molar relationship in the variations of the two substances.

Also the phosphocreatine decrease is not proportional to the rise of lactic acid in the stage of acute injury, although there is a general tendency toward this inverse relationship. Similar lack of proportion was found by Olmsted, Mackler, and Simpson[11] who state that in stimulated frog gastrocnemii there is much greater accumulation of lactic acid than can be neutralized by the amount of phosphocreatine hydrolyzed.

Pekelharing and Van Hoogenhuyze[12] found an increase of creatine in the

muscles in rigor, and in increased tonus produced in Sherrington's decerebrate rigidity, which might be accounted for now by an increased hydrolysis of phosphocreatine in the muscles. Creatine has for many years been associated in the literature with muscle function, and generally considered to have to do with tonic functions of muscle. For example, Pekelharing[13] found an increased creatinine excretion in men with increased tonicity of muscles caused by long standing erect at attention, or by long continued electrical stimulation, without contraction. Exercise for the same period of time did not give increase of creatinine excretion.

Inorganic phosphorus likewise shows a tendency toward an inverse relationship to phosphocreatine, in that it is generally higher than the control value during the period of repair and sinks toward the end of that period. In active and resting muscles variability of inorganic phosphate may be due to the breakdown and reformation of hexose phosphate. Irving and Bastedo[14] found variable amounts of inorganic phosphorus formed in exercised muscle in relation to lactic acid and phosphocreatine.

SUMMARY

In acute molecular degeneration of striated muscle in rabbits, there is a sudden rise of lactic acid which reaches a high point in four hours. Thereafter it declines, rapidly at first, and later more gradually to about normal value toward the end of the observation period of twenty-eight days.

Glycogen drops to a low point within forty-eight hours, but its decrease shows no close ratio to the amount of lactic acid formed. The later increase in glycogen during repair brings it very close to the control range.

Phosphocreatine decreases rapidly, while inorganic phosphorus increases somewhat late and irregularly. This inverse relationship persists through the repair stage, with a slowly rising phosphocreatine value, and a very irregular inorganic phosphorus which tends toward a decreased level.

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STUDIES OF EXPERIMENTAL MUSCLE DEGENERATION*

V. Nitrogen Metabolism of the Degenerated Muscle in Acute Injury and Repair

D. K. Fishback, Ph.D., and H. R. Fishback, Sc.D., M.D., Chicago, Ill.

THE skeletal muscles make up the largest mass of protein in the animal body. Many nitrogenous substances are required by them, and yet an understanding of the use of these substances in muscle metabolism has been most difficult to work out. It is assumed that muscle cytoplasm is built up from the amino acids of the blood, and the nucleo-protein partly from blood purines. Creatine has long been associated with muscle tone[1, 2] although its exact relationship to this phenomenon has not been understood. The full rôle of creatine in muscle economy is perhaps not yet completely known, although its important association with carbohydrate metabolism through its combination with phosphoric acid has been presented, by Fiske and Subbarow[3] and Eggleton and Eggleton.[4] Urea was formerly thought to be formed in part in the muscles, until the demonstration by Bollman, Mann, and Magath[5] that this function is performed by the liver only. An important chapter, added by Embden[6] and Parnas[7] is the formation of ammonia in muscle by the deaminization of adenine nucleotide, with the formation of inosinic acid. Several other nitrogenous substances are grouped as muscle extractives, without having as yet any ascribed function or, in some cases, without being properly vouched for as constituents of living muscle.

But little attention has been paid, as yet, to the metabolism of nitrogen compounds in abnormal muscle in the animal. In fatigued and in ischemic muscles the study of creatine in relation to phosphates and carbohydrates proved a marked drop in creatin-phosphoric-acid.[8, 9] Muscle ammonia is increased in amount on removal of the muscle from the body, and Parnas[10] states that, in the animal muscle, ammonia is increased in those conditions, such as asphyxia, fatigue, and rigor, which lead to increased lactic acid formation. Such ammonia increase arises, as in normal metabolism, from deaminization of adenine nucleotide. The decrease of adenine is practically balanced by an increase of hypoxanthine.[7] The inosinic acid formed is further decomposed by the splitting off of phosphoric acid.

No report has been found in the literature on the nitrogen changes in healing injury of muscles.

Experimental.—In the present work, chemical studies were carried out on rabbit muscles injured and prepared for analysis according to a procedure pre-

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viously described.[11] The animals were sacrificed at varying lengths of time after injury, up to twenty-eight days, in order to study the changes associated with repair of the injury. Microscopic studies were made on all the muscles removed and are reported in another place.[12] Total nitrogen determinations were made by the Kjeldahl method on 1 gm. samples or larger. In the trichloracetic acid filtrate total nonprotein nitrogen and urea nitrogen were determined according to Folin and

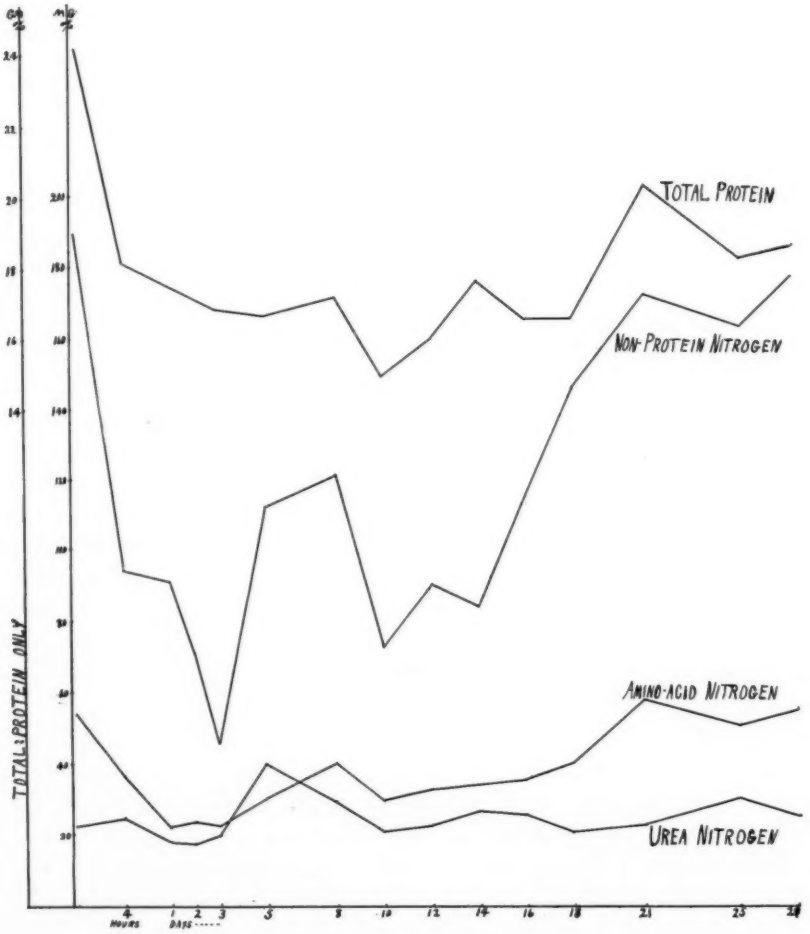

Fig. 1.—Nitrogen metabolism during muscle repair.

Wu,[13] amino-acid nitrogen by Folin's method,[14] and phosphocreatine by the method of Fiske and Subbarow.[8] All determinations were made in duplicate.

Results.—Results are presented graphically in Fig. 1.

DISCUSSION

The total nitrogen values have been converted to protein by multiplication with the factor 6.25. The protein content of the muscle is found to be decreased during the time of the period of healing. It is probable that the early fall in protein content is influenced to some extent by edema. The digestion of the proteins

of dead muscle cells either by autolysis or by ferments of inwandering cells might also alter the protein mass. However, there is no evidence of such digestion in either the nonprotein or amino-acid nitrogen values, since both of these run considerably below control values during the stage of acute muscle injury. On microscopic study of the injured muscles the greater number of exudative cells are found to be mononuclears, many of which are phagocytic.[11] The removal of protein by phagocytosis would give no chemical evidence of the process except in decrease of the total protein value.

The amino acids of the muscles, according to Van Slyke and Meyer,[15] may serve as a reserve, either for energy supply or tissue building. Also they may be intermediate products of tissue building or of tissue break-down. Their origin in the muscle might be from either the ingested food or muscle protein decomposition. Since in our work the amino acids are markedly decreased in the stage of acute injury, and rise slowly only toward the end of the period of observation, there is no indication of break-up of the muscle protein into its component chemical units. If amino acids do serve as a reserve for energy or tissue building, it is likely that sufficient amounts are continually being brought in by the blood, so that there is no need for any marked storage in the tissues. No increase over normal was observed during the stage of active cell proliferation, when an excess of amino acids might have been expected to be on hand for use as the building-stones of new protein molecules. Collip[16] suggested a further possible function for amino acids in maintaining osmotic pressure in the cell, and especially in the nucleus, which has no inorganic electrolytes.

The value for urea nitrogen in the injured muscles fluctuates somewhat throughout the entire observed healing stage, from the value in the control animals. The values given for urea nitrogen include also ammonia nitrogen, which in normal muscles is so small in amount in comparison with the urea nitrogen value as to be negligible.[10] In a condition such as heat rigor, however, there is an increase of ammonia nitrogen from a control of about 0.5 mg. per cent up to about 7.3 mg. per cent.[17] This increased ammonia nitrogen is equal to about 20 to 30 per cent of the corresponding urea nitrogen. With such possibility of ammonia formation in muscle injury, it is likely that our urea nitrogen figures as given are too high by the amount of possible ammonia nitrogen increase. Since the decisive work of Mann, Bollman, and Magath,[5] the liver has been accepted as the sole seat of urea formation in the animal. A few workers have been unable to demonstrate urea formation in muscle digests.[18, 19] The lack of constant increase of urea nitrogen in our experiments indicates that, in this type of acute molecular degeneration, injured muscles do not have the power in vivo of forming urea. The holding power of the injured muscle for urea is likewise but little altered.

Creatine has been determined not as a separate value but as the phosphoric acid compound which is important in carbohydrate metabolism, and is considered in a paper on that subject.[20] It was found to be markedly decreased in amount quite early after the injury, with a gradual rise during the period of repair.

The nonprotein nitrogen might be expected to vary in the same muscle with different protein precipitants, which release somewhat different amounts of soluble nitrogen. Trichloracetic acid in 5 per cent solution, which was used uniformly

in our experiments, precipitates proteins but allows a maximum of amino acids and intermediate protein split products to remain in the filtrate.[21] It thus extracts protein products of lower grade and likewise other nitrogenous extractives of the muscle not bound to protein in acid solution. The total value of such muscle extractives in control animals averages 187 mg. per cent of nitrogen. In the acute injury stage, the value drops rather rapidly to a low point of 45 mg. per cent in three days. The recovery is likewise rather rapid, although the value fluctuates considerably, reaching almost to the control level toward the end of the observation period.

It is rather striking that these values indicate no autolysis of the degenerated muscles. Some factor associated with the vitality of the tissue appears to be active in protecting the degenerated muscles in vivo against the action of autolytic ferments.

Microscopically the rapid growth of cells for repair matches the rapid rise toward normal of nonprotein nitrogen in the early stage of repair.[11] The supply of nitrogenous food brought by the blood thus appears to be ample to support rapid and continuous formation of the cytoplasm and nuclei of new cells, although apparently no extra supply above the normal is needed.

SUMMARY

In acute molecular degeneration of striated muscle in rabbits, the decrease of total nitrogen is roughly proportional to the edema, the value rising slowly with subsidence of the edema.

The nonprotein nitrogen value falls sharply with the acute injury and rises rather abruptly in the early stage of repair, reaching almost to the control level toward the end of the observation period of twenty-eight days.

Urea nitrogen varies inconstantly throughout the acute injury and stage of repair. There is no evidence that urea is formed at all, or that it is held in increased amounts, in such injured muscles in vivo.

The amino acid value of the degenerated muscle is reduced markedly but rises during the stage of repair to the normal range.

There is no evidence presented of autolytic break-down of the muscle mass.

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A COMPARISON OF THE EFFECTS OF MORPHINE, PANTOPON, CODEINE, NARCOTINE, AND PAPAVERINE ON THE RESPIRATION OF RATS AND RABBITS*

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IN SPITE of the rather general agreement among early observers as to the effects of the individual opium alkaloids on respiration, the literature on the pharmacology of various combinations of the alkaloids as compared with morphine is quite contradictory, especially in so far as the toxic, narcotic, and analgetic properties are concerned. The state of our knowledge in this respect is reflected by the statement of Leake (1930), that "little rational evidence exists which would indicate that opium or a mixture of its alkaloids, free from nonalkaloidal material, has any advantage over morphine."

In order to determine the efficiency of the several agents as to their sedative or tranquillizing action as well as for preanesthetic medication, it was deemed necessary to repeat certain of the older studies of the effects of morphine and related compounds on respiration for purposes of correlation with the above observations. The effects of the more important opium alkaloids and pantopon on the respiration of the rat and rabbit are reported in the present study.

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Methods.—With the exception of morphine, no data on the effects of the opium derivatives on the respiration of the rat are available, possibly because of the size and peculiarities of this animal. The method described by Barbour and Maurer (1920) for measuring the actual expiratory volume of the rat necessitated limitation of the animal's movements, the use of water valves and the insertion of the head in a mask. Results obtained by us with this procedure were so variable that it was discarded.

The procedure followed throughout this study consisted of tying the animals on their backs on specially adapted boards, attaching a lever by means of a thread to a point on the abdomen just below the xiphoid process and recording the respiratory movements on a moving drum. This restraint places the animals under uniform but decidedly abnormal conditions which render the rats more restless than normal. This restlessness is a decided advantage for studying the effects of sedatives.

The data obtained by this method are entirely satisfactory as to the respiratory rates (checked by means of a stop watch) but the volumes recorded are only indirectly representative of the chest movements and are complicated by occasional struggling of the animals. However, the results were sufficiently constant to permit a rather close comparison of the effects of the several agents on the rate and qualitative values of volume. Graphic records were obtained over three satisfactory ten-minute periods during the alternate inhalation of 100 per cent oxygen, and of 95 per cent oxygen, and 5 per cent carbon dioxide before and again forty-five to sixty minutes after, the drug administration.

All drugs were dissolved in distilled water. The concentration in each case was so adjusted that the volume injected (subcutaneously) was as nearly constant as possible. The HCl salts of morphine, papaverine and pantopon and the H_2SO_4 salts of narcotine and codeine were used. The dosages in each case were computed as the base. The basis of equivalence on which morphine was compared with the other agents tested is that usually accepted clinically, i. e., 1 grain of morphine equals 1.66 grains of pantopon, 2 grains of codeine, or 4 grains of papaverine.

RESULTS ON RATS

A series of at least 5 (5 to 15) rats were used for every dosage of each of the drugs tested. The normal respiratory rates per minute and the volume (measured excursion of the recording lever in millimeters) was considered as 100 per cent for purposes of statistical comparison with the reactions after medication. The deviation of the rates and volumes from the control values were then computed as percentages of the respective controls. The tabulated data have been expressed as medians in the several illustrations.

Morphine.—The respiratory changes were noted following the administration of dosages of morphine ranging from 2.1 to 63 mg. per kg. (½ to 15 per cent of the M. L. D. considered as 0.42 gm. per kg.) of rat. *The rate response* with small dosages (2 to 3 mg. per kg.) varied within a range of 8 per cent, while larger dosages were purely depressant. The median changes noted with all dosages tested are illustrated in Fig. 1, which shows that the degree of depression increases rapidly with dosages up to 15 mg. per kg. and more gradually as the dosage was further

increased. The depression following the administration of the maximal dosage tested was only 3 per cent greater than that observed with 21 mg. per kg. although the actual difference in dosage was 300 per cent. *The respiratory volume* diminished progressively with increasing doses over the range tested. The degree of change was negligible with 2.1 mg., increased very gradually and became maximal (50 per cent) following the administration of the maximal dosage (Fig. 1). *The minute volume* as calculated by multiplying the rate per minute by the measured excursions in millimeters, was depressed by all dosages of morphine tested. The depression was minimal (9 per cent) with 2.1 mg. per kg., increased rapidly with moderate, more gradually with larger dosages and became maximal (65 per cent) following the administration of 63 mg. per kg.

Pantopon.—The effects of morphine and pantopon (the mixed opium alkaloids) were compared on the basis of weight, therapeutic equivalence, and the morphine alkaloid content, i. e., 1 grain of morphine equals 2 grains of pantopon.

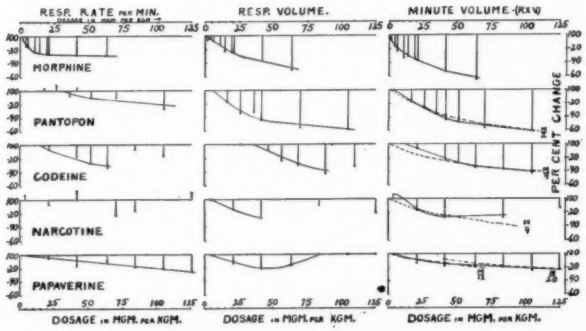

Fig. 1.—This figure represents the effects of morphine, etc., on the respiratory rate, volume, and minute volume of the rate. The dosages in milligrams per kilogram of animal are represented on the abscissae. The changes in terms of percentage of the original or normal observations are indicated on the ordinates. Morphine has been superimposed as the broken line (min. vol.).

The effects of pantopon on the respiration could not be differentiated from those of the morphine content of the dosages administered (Fig. 1). *The variations in rate* after small to medium doses were capricious and smaller in degree than after comparable doses of morphine. However, the differences noted between morphine and pantopon in this respect cannot be explained on the basis of statistical error as volume changes largely compensated for the irregularities in rate, and the calculated minute volumes diminish quite regularly with increasing dosages. This relation is illustrated in Fig. 1. Superimposing the curve for the minute volume changes under morphine on that for pantopon by dividing the morphine data by 2, indicates the negligible differences between the two data. The differences are probably within the range of experimental variation. The minor alkaloids of pantopon therefore, do not significantly alter the effects of the morphine contained as concerns respiration. On the basis of therapeutic equivalence, the depressant effects of pantopon were somewhat less than those of morphine but on the basis of morphine equivalence, the two agents were indistinguishable; i. e., pantopon (which contains 50 per cent morphine) was almost exactly one-half as depressant as an equal weight of morphine.

Codeine.—The effects of codeine on the respiration were studied with doses ranging from 21 to 105 mg. per kg. of rat. The depressant effects of small to medium doses were less than one-fourth that of equal weights of morphine. However, with large but subconvulsive doses the degree of depression corresponded very closely to that of a one-fourth dose of morphine (broken line superimposed on codeine minute volume curve). With toxic dosages of codeine (above 105 mg. per kg.) the depression of rate and volume diminished, presumably by the development of the convulsive action of codeine.

Narcotine.—The respiratory effects of narcotine are relatively unimportant, although a moderate depression was noted with all dosages tested (Fig. 1). *The rate changes* were variable while *the volume* was uniformly depressed. The depression increased gradually to a maximum with 42 mg. per kg. of rat and either showed little further change or actually diminished. The reactions to small to medium doses were approximately one-fourth those of equivalent doses of morphine, but with larger doses the narcotine depression decreases and as the convulsant action becomes increasingly evident, the depressant effects are overshadowed.

Papaverine.—The effects of papaverine in the dosages tested were purely depressant. *The rate* diminished progressively with increasing doses. *The volume* also diminished with dosage up to 42 mg. per kg. but with larger doses the depression largely or completely disappeared. *The changes in minute volume* following the administration of doses up to 60 mg. per kg. were approximately equivalent to one-twelfth those of similar dosages of morphine. However, in dosages above 75 mg. per kg., papaverine was only one-twentieth as depressant as morphine.

COMPARISON OF THE STIMULANT EFFECTS OF 5 PER CENT CO_2 ON THE RESPIRATION BEFORE AND AFTER MEDICATION WITH MORPHINE, ETC., WITH RATS

In tabulating the changes in rate, volume, and minute volume following inhalation of 5 per cent CO_2, the control values noted during the inhalation of oxygen were considered as 100 per cent both before and after medication. The data show the degree of stimulation in percentages of the preceding oxygen control. The legends are self-explanatory.

With drugged rats, the inhalation of 95 per cent oxygen and 5 per cent carbon dioxide following exposure to 100 per cent oxygen, resulted in an increase in rate, volume, and minute volume. The increase in minute volume varied from 80 to 200 per cent above normal with different series of animals with a median increase of 130 per cent for all animals tested. The median increase in rate was 48.5 per cent and that of volume 62.4 per cent. The medians of the entire series are indicated as the normals (marked as X on the ordinates) in all figures. Animals whose normal responses varied by more than 25 per cent from these medians were discarded.

Morphine.—The stimulant effects of CO_2 on the rate, volume, and minute volume after medication with various doses of morphine are indicated in Fig. 2. All doses tested reduced the respiratory response to CO_2. *The rate reduction* with small to moderate doses was distinctly greater than the accompanying volume changes. With larger dosages, the rate and volume changes were fairly commensurate. The depression of the rate response increased rapidly with small to me-

dium but more gradually with larger dosages. With 2.1 mg. per kg. the depression was minimal. The degree of depression with 5 mg. was approximately one-third and with the maximal dosage (63 mg. per kg.) eight-tenths of that observed in the absence of medication. *The volume* was reduced by all dosages tested; but the marked rate reduction noted with small to moderate doses of morphine was partially compensated for by a relatively smaller effect on the volume. *The depression of minute volume* increased rapidly with small to moderate and more gradually with larger dosages. After medication with 21 mg. per kg. (the minimal tranquillizing dose), the degree of stimulation was 42 per cent and after 63 mg. per kg. only 20 per cent of that noted before medication.

Pantopon.—The stimulant effects of CO_2 on the respiratory rate and volume after medication with pantopon (Fig. 2) appeared to differ in certain respects from those noted after comparable doses of morphine. *Small doses were slightly less and larger doses more depressant to the rate* than morphine. *The volume response* was reduced somewhat with small doses but differed little from the control values

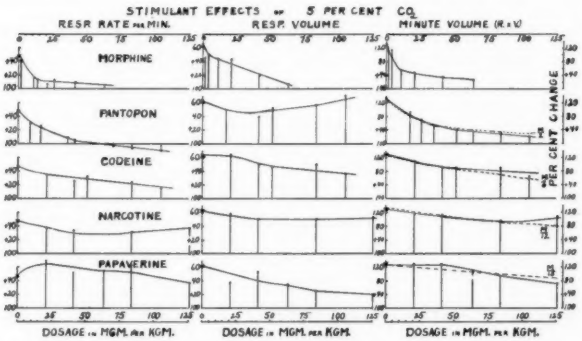

Fig. 2.—This figure indicates the stimulant effect of 5 per cent CO_2 after medication with the several agents. The control data after medication was considered as 100 per cent. The curves indicate the change from this medicated level.

after larger dosages in contrast to the reaction noted after morphine. However, the rate and volume changes were compensatory in that the apparent differences between the reactions to pantopon and morphine disappear when the calculated minute volumes are compared, and the curves for pantopon and its morphine content (broken line) are practically identical. It appears probable therefore that the observed differences in rate and volume may have been due to accidental variations in the compensatory adjustment of the animals.

Codeine.—The stimulant effect of CO_2 on the respiratory rate was reduced by codeine. *The degree of rate change* increased gradually with increasing dosage to a maximum of approximately 60 per cent with 105 mg. per kg. *The volume response* was likewise reduced. However with dosages greater than 50 mg. per kg. little further depression occurred and the median reactions with large doses were actually greater than before medication. Correlation of the stimulant effects of CO_2 on the minute volume following medication with codeine and morphine (broken line) indicates that small to medium doses of codeine are approximately one-sixth, while larger dosages are distinctly less than one-sixth as depressant as morphine.

Narcotine.—All dosages tested reduced the normal rate response to CO_2. The respiratory volume shows but little in the median figures and the individual experiments exhibit a greater degree of stimulation after than before medication, but the degree of increased response diminished with increasing dosages. The minute volume response to CO_2 was also invariably greater than normal after narcotine. The stimulant effect of this agent increased to a maximum with a dosage of 42 mg. per kg. and decreased with larger dosages so that no significant change was evident with a dosage of 126 mg. per kg. These data appear to indicate that narcotine in small to moderate doses stimulates the respiratory center. However a depressant action is evident as the degree of stimulation decreases with further increase in dosage.

Papaverine.—The respiratory rate response to CO_2 following medication with papaverine in doses up to 105 mg. per kg. was greater than in the absence of medication. *The volume response* on the other hand was progressively reduced with increasing dosage. *The minute volume,* on inhalation of CO_2, was distinctly increased above the control values with doses up to 42 mg. per kg. but further increase in dosage resulted in a progressive depression of the normal response. Papaverine in moderate dosage appeared to stimulate the respiratory center. Large doses (up to 84 mg. per kg.) are approximately one-fifteenth, while higher dosages are more than one-twelfth as depressant as equivalent weights of morphine.

RESULTS ON RABBITS

The procedures followed in the preceding section were repeated on rabbits in order to compare the respiratory responses of the two species to similar experimental conditions as well as to check the results obtained with those reported in the literature. In addition, the actual minute volumes were measured during the inhalation of O_2 and of a mixture of 95 per cent O_2 and 5 per cent CO_2, both before and after medication with representative doses of the several agents. Measurements of the minute volumes were obtained by tying the animals in a dorsal position, the insertion (under local anesthesia) of a tracheal cannula in order to eliminate the possibility of leakage encountered with the mask method, the use of water valves for control of the air intake and output, and measurement of the expiratory volume by means of a Bohr gas meter. Each 250 c.c. of the expired air was recorded electrically on a moving drum.

Morphine.—The respiratory rate was reduced by all dosages tested (Fig. 3). The depressant effects were evident following the administration of the minimal dosage (0.26 mg. per kg. or 0.1 per cent of the M. L. D.) and increased rapidly with dosage so that the reduction was 80 per cent of the original after medication with 2.6 mg. per kg. The degree of depression increased slightly with larger dosages. *The volume* was reduced by 10 to 25 per cent with dosages between 0.26 and 1.3 mg. per kg. but was practically unaltered by larger doses in the range tested probably because of a partial compensation for the marked alteration of the rate. *The minute volume* was actually increased by the minimal dosage tested although the rate and volume changes illustrated in the figures (obtained graphically) indicate a mild depression. With larger doses, the degree of depression of the minute volume increased with the dosage administered.

Pantopon.—The rate changes following the administration of pantopon differed in no significant respect from those produced by equivalent dosages of morphine. *The respiratory volume* was diminished by small doses to approximately the same degree as by morphine. With moderate dosages, the volume was increased slightly, thus partially compensating for the distinct accompanying rate depression. After maximal doses, the volume changes were indistinguishable from those of the morphine contained in the preparation. *The minute volumes* following the administration of small doses of pantopon were more variable than after morphine but the median values were practically the same. Larger doses were somewhat more depressant than morphine but the differences noted probably lie within the range of error and cannot be considered as significant.

Codeine.—The respiratory rate of the rabbit was depressed by all doses of codeine tested (2.6 to 26 mg. per kg.). This effect increased with the dosage administered, but the maximal reduction observed was only 20 per cent of the normal or initial readings. Small doses were approximately one-fourth while larger doses

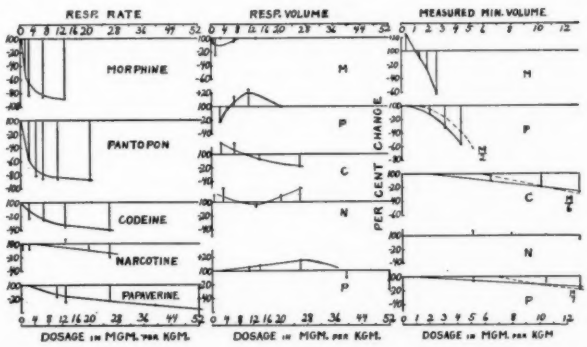

Fig. 3.—This figure represents the same data for the rabbit as Fig. 1 for the rat. However the minute volume in Fig. 3 represents the actual gas meter measurements of the changes observed.

were less than one-sixth as depressant as equivalent doses of morphine. *The volume* was increased approximately 15 per cent with dosages below 6.5 mg. per kg. Larger doses were depressant but the maximal effect noted was only 15 per cent of the original values. *The minute volume changes* were insignificant with small to medium dosages although depression was the rule. The reduction observed with the maximum dosage tested was less than one-sixth that of equivalent dosages of morphine.

Narcotine.—The respiratory rate following the administration of narcotine although variable was primarily depressed. The degree of change ranged from 5 to 8 per cent with small to moderate and increased with larger dosages. The depressant effect of narcotine was less than one-eighth that of morphine. *The volume* was primarily increased with all dosages. The degree of stimulation varied from 10 to 20 per cent of the original volume. This reaction was quite unlike that of morphine and was comparable to the effects produced by moderate dosages of codeine. No significant alteration of *the minute volume* was observed. Small doses were slightly stimulant while larger dosages were either ineffective or slightly depressant. The changes noted although suggestive, are within the range of normal variation.

Papaverine.—The respiratory rate was depressed by all dosages tested. The degree of depression increased gradually with the dosage administered and ranged from 20 to 50 per cent of the normal. *The volume* on the other hand was increased gradually with dosages up to 26 mg. per kg. Larger doses were somewhat more depressant than morphine. *The calculated minute volumes* following the administration of papaverine were uniformly depressed. The degree of change varied from 5 to 18 per cent over the range of dosages tested. The depressant effects of papaverine on the respiration of the rabbit were about one-seventh that noted with equivalent doses of morphine.

THE EFFECTS OF PANTOPON AND THE SEVERAL OPIUM ALKALOIDS ON THE RESPIRATORY RESPONSE TO CO_2

The respiration of the rabbit under the conditions of these experiments was quite inefficient in that a rapid rate and a correspondingly shallow volume was observed. The inhalation of 5 per cent CO_2 and 95 per cent O_2 resulted in a distinct

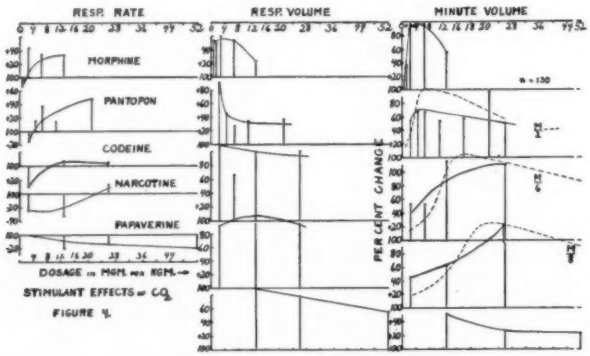

Fig. 4.—This figure represents the same data for the rabbit as Fig. 2 for the rat.

decrease in the rate and a more than compensating increase in the volume so that the average minute volume was increased by 50 to 80 per cent above that noted during the inhalation of 100 per cent O_2.

Morphine.—The usual rate and volume responses to CO_2 were distinctly altered following the administration of morphine, i. e., the rate was increased and although the volume was increased the degree of change was distinctly less than in the absence of medication. This altered reaction is quite understandable when the effect of morphine alone is recalled, i. e., a marked depression of rate and a relatively slight alteration of volume. In Fig. 4, the respiratory rates and volumes after medication were considered as 100 per cent in order to illustrate the relative stimulant effects of CO_2 on the depressed center. Under such conditions the percentile change was distinctly greater after than before medication although the absolute minute volumes after medication diminished progressively with increasing dosages. The disparity between the reactions to CO_2 was apparent with the minimal dosage tested, increased to a maximum with 2.6 mg. per kg. and diminished with larger doses. Measurements of the actual minute volumes likewise indicate that the stimulant effects of CO_2 are proportionately greater after than before medication.

Pantopon.—The respiratory reaction to CO_2 after medication with pantopon was similar and did not differ significantly from that described for the one-half dosage of morphine under similar conditions.

Narcotine, Papaverine, and Codeine.—The rate and volume changes which occurred on inhalation of CO_2 following medication with these agents differed in certain respects from those noted after morphine, in that the rate increase was smaller in degree and the volume was increased rather than reduced as in the case of morphine. These differences are explained by the disparity between the degrees of respiratory center depression produced by morphine on the one hand and by these minor alkaloids on the other. In spite of the observed sedative effects of codeine, papaverine, and narcotine on respiration, the sensitivity of the center to CO_2 was practically unaltered and in the case of narcotine an apparent stimulation occurred.

DISCUSSION AND SUMMARY

Morphine.—The respiratory changes noted in the rat following the administration of morphine differ only in minor respects from those noted by Barbour and Maurer (1920). Small doses may increase the rate slightly although a mild depression is the rule. Medium doses produce a progressive depression. The volume was reduced by all dosages tested. However, the degree of change bore only a qualitative relation to the dosage administered. The minute volume was decreased by all dosages tested and the degree of change increased gradually with dosage so that with 63 mg. per kg. the gas exchange was only 35 per cent of normal.

The respiratory changes noted in the rabbit do not differ in any important respect from those noted by Gscheidlen (1869) or Impens (1899). Minimal doses affected the rate to a negligible degree. The depression was reflected primarily by alterations in rate in contrast to the nearly equal changes which occurred in both rate and volume in the rat. The volume was increased slightly by small doses, practically unaffected by medium to large and markedly depressed by toxic dosages. The actual minute volumes were increased with doses of 0.26 mg. per kg. (equivalent to 0.1 per cent of the M. L. D. for the rabbit). The minimal depressant dosage was approximately 0.65 mg. per kg. body weight.

The depressant effects of morphine on the rat and rabbit are quite comparable when only changes in minute volume and the differences in the sensitivity of the two species to this alkaloid are taken into consideration. The degree of depression produced by the maximal dosages tested (i. e. 13 mg. in the rabbit = 5 per cent of the M. L. D., and 63 mg. per kg. in the rat = 15 per cent of the M. L. D.) was the same (65 per cent) in each species.

The respiratory changes which occurred following the administration of narcotine, codeine, or papaverine differ only quantitatively from those noted for morphine, as reported by v. Merck (1848), v. Schroeder (1883) and Meissner (1913). Only quantitative differences were noted between the responses of the two species. Straub (1912) reported that narcotine alone was ineffective, but Möhrke (1921), and Rikl (1927) observed an increase in respiration in the presence of this alkaloid.

The depressant effects of codeine increased gradually to a maximum and subsequently decreased with larger doses as the reflex excitability increased. The depressant action of papaverine increased gradually to a maximum with medium

doses but further increase in dosage produced little further depression. The reaction to narcotine was of little significance.

On the basis of the degree of depression produced by the several agents tested, codeine in small to medium dosages was approximately one-fourth, narcotine in small to moderate doses was one-fourth and in larger dosage less than one-eighth, while papaverine in doses up to 63 mg. per kg. was one-twelfth and in larger dosages approximately one-twentieth as depressant as morphine.

A direct comparison of the respiratory effects of morphine and pantopon on rats and rabbits indicates that the effects of the two agents differ only quantitatively although the responses to small doses of pantopon were more variable than after morphine. The significance of these minor differences is questionable. The pantopon effects may be entirely explained on the basis of the morphine contained. No evidence was obtained which would indicate that a potentiation of the effects of morphine occurs in the presence of narcotine as reported by Sahli (1910), Straub (1912), or Möhrke (1921). The effects produced by a combination of the residual alkaloids of opium with morphine probably represent a simple summation of the several actions as claimed by Wertheimer-Raffalovich (1910), v. Zeelen (1911), Bürgi (1912) and Meissner (1913).

The depressant effects of the several agents on the respiratory center as illustrated by the stimulant effects of 5 per cent CO_2 before and after medication differ only quantitatively and parallel in general the changes which occur in the rate, volume, and minute volume, with the exception of narcotine and large doses of codeine. Narcotine apparently sensitizes the respiratory center to CO_2 in that the stimulant effects of this gas after medication with this alkaloid were greater than normal. It is quite probable, however, that the effects of narcotine are due to both stimulation and depression since the stimulant action of CO_2 diminished markedly with increasing dosages.

The efficiency of morphine, pantopon, and codeine as antidyspneic agents is probably illustrated by their effects on the respiratory reaction to CO_2. The sensitivity of the respiratory center to CO_2 was reduced by all dosages of morphine or pantopon. However, in therapeutic dosages, the changes in ordinary respiration were not significant especially when the more efficient type of respiration which occurs after such medication is considered. Codeine should prove to be of value.

Toxic dosages of morphine or pantopon markedly depress ordinary respiration and distinctly decrease the sensitivity of the center to CO_2. Codeine and papaverine in large dosages were also slightly depressant but in the main, codeine, papaverine, and narcotine were convulsant in toxic dosages and the respiratory depression was of little significance.

CONCLUSIONS

1. The respiratory changes which occur in the rat and rabbit following medication with morphine, pantopon, codeine, narcotine and papaverine have been studied. Little difference was observed between the responses after medication when the difference in species sensitivity was taken into consideration.

2. On the basis of the degree of depression produced by the several agents, pantopon was one-half, codeine in small to medium doses one-fourth, narcotine one-fourth to one-eighth and papaverine one-seventh as depressant as morphine.

3. The depression of the respiratory center by the series studied parallels in general the observed changes in minute volume, with the exception of narcotine and large doses of codeine. Narcotine apparently sensitizes the center to CO_2 but this effect decreases with increasing dosage.

4. The residual alkaloids of opium in the proportion present in pantopon do not alter the usual respiratory response to morphine to any remarkable degree. The respiratory effects of pantopon may be explained on the basis of its morphine content.

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CHANGES IN DISTRIBUTION RATIO OF CONSTITUENTS OF BLOOD AND SPINAL FLUID IN MENINGITIS*

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IN THE study of the changes in the spinal fluid during the progress of meningitis, it must be borne in mind that all the abnormal reactions of the tissues which constitute the investing membrane of the brain and spinal cord, and to some extent the tissues of the brain itself, may be produced by different causal agents brought to the parts by diverse channels; in other words, the invading microorganism and the mechanics of invasion may differ in various types of meningitides, but the effects are characteristically similar in the tissues affected, and they produce symptoms common to the involved tissues but modified to some extent by the nature of the invading organism and the number actually introduced. We have endeavored in this work to trace some of the common characteristics of the disturbances of physicochemical equilibria which result from the pathologic state, in order, if possible, to arrive at some definite chemical changes which may aid us in diagnosing at an early date the signs of meningeal involvement.

Animal experiments carried out by Kopetzky,[1] in which the intracranial pressure was artificially increased, showed that anemia of the brain could be produced and that the increased amount of cerebrospinal fluid then formed in the intracranial spaces resulted in a compressed blood supply, with interference with both the afferent and efferent vessels; and hence resulted in a lessened oxygenation of the blood in this region. It has been shown by Meyerhof and Warburg that the result of lessened oxygen tension is an incomplete oxidation of carbohydrate, so that the so-called anaerobic type of oxidation preponderates over the aerobic, and large quantities of lactic acid are produced. This phenomenon can be seen in the blood during fever, in certain forms of heart disease, and in cases of heavy and sudden exertion, where the rate of oxygen supply becomes insufficient for the total combustion of the sugars, the carbon dioxide and water.

There is an increased amount of lactic acid in the spinal fluid in meningitis. The source of this lactic acid is as yet doubtful, but it is certain that the factors above mentioned tend to increase its concentration in the spinal fluid. There have been several theories that the increased lactic acid was a result of the fermentation of the sugar, which disappeared from the spinal fluid during the progress of many cases of meningitis. That this factor also may play a rôle in some cases cannot be denied, but we have, as was also shown by Killian,[2] found in certain cases a much increased lactic acid in the presence of a practically normal spinal fluid sugar content. There is a possibility that the disintegration of the leucocytes in purulent meningitis may be a source, because Levene and Meyer[3] have shown that leucocytes

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suspended in Henderson's phosphate mixture containing glucose produce glycolysis with formation of lactic acid. Again, it is a known fact that the embryonic cells of the choroid plexus have an active glycogen metabolism, so that it is not without the realm of probability that the cells of the choroid plexus may be able to actually secrete the lactic acid and concentrate it from the blood, a function that has lately been shown by one of these authors to be characteristic of the skin.

In normal spinal fluid there is a definite ratio for the relationship of spinal fluid lactic acid to blood lactic acid which never exceeds 1.0, and is usually approximately 0.6. This may even be lower in cases of exertion, fever or heart disease. In such cases, due to the presence of increased lactic acid in the blood, and an absence of specific inflammatory condition in the meninges, the ratio is lower because of the

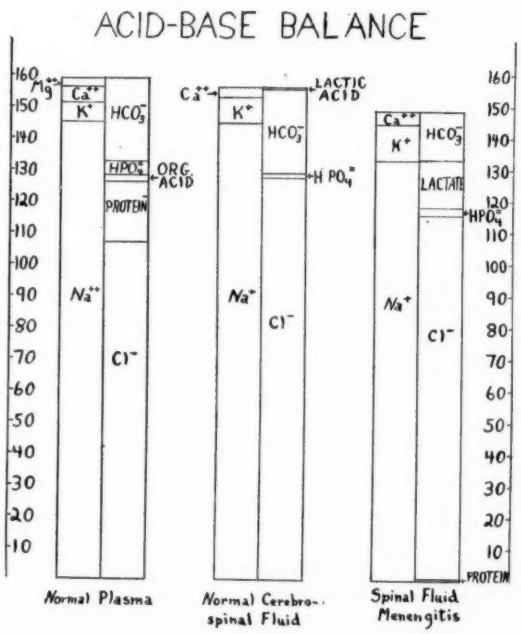

Fig. 1.

increased blood lactic acid. In cases of true meningitis, this ratio may rise to 3 or 4 during the progress of the disease. This effect was noted by Glaeser[4] and confirmed by Killian.[2] Glaeser found, that in two cases of epidemic spinal meningitis, the lactic acid in the spinal fluid rose to more than 150 mg. per 100 c.c. This would give a ratio of over 5. In our cases as can be seen from Table I the range was between 1.5 and 3.5.

The increased lactic acid content of the spinal fluid results in several interesting consequences. The lactic acid, being a relatively strong acid, drives off CO_2 from the carbonates, and hence, in cases of meningitis we should expect to find on chemical analysis of the spinal fluid a considerably lowered P_H, a decreased bicarbonate content and an increased lactic acid. This is found to be exactly the case.

Another effect of the increased lactic acid found in spinal fluid in meningitis can be predicted on theoretical physical chemical grounds, which experimental re-

TABLE I

TYPE OF CASE	P_H	CO_2 VOLUME PER CENT	CO_2	LACTIC ACID		PROTEIN MG. PER 100 C.C.	Na C.C. N/10	Na MG. PER 100 C.C.	K C.C. N/10	K MG. PER 100 C.C.	BLOOD LACTIC ACID MG. PER 100 C.C.	RATIO SPLA/BLA	CL MG. PER 100 C.C.	CL C.C. N/10	BLOOD PLASMA CL MG. PER 100 C.C.	BLOOD PLASMA CL/SPINAL FLUID GRADIENT MG./100 C.C.
				MG. PER 100 C.C.	C.C. N/10											
Strep. hem. meningitis	6.9	49.0	22.2	22	3.6	208	132	304.7	9	17	20.9	1.05	350	98.5	348	+2
Strep. hem. meningitis	6.9	50.2	22.6	54	6.0	202	122	281.7	7.5	14.2	28	1.92	340	93.0	364	−24
Meningitis	6.9	43.8	19.8	120	13.3	144	145	333.5	14.1	27	34	3.54	372	102	356	+16
Meningococcus meningitis	6.9	47.8	21.4	34	3.9	94	137	310.5	11.2	21.3	21	1.62	381	107	361	+20
Tb. meningitis	7.1	44.2	19.8	84	9.3	235.6	127	293	14.7	28	28	3.00	362	102	364	−2
Tb. meningitis	7.3	51.9	22.9	84.5	9.4	252	137	316	11.2	21.3	26	3.23	354	100	359	−5
Tb. meningitis	7.4	56.6	25.4	84	9.3	267.7	144	332	12.6	24	28.1	2.80	369	102	363	+6
Meningitis mumps	7.0	43.6	19.6	75.5	8.4	118	136	314		18	29	2.6	341	99	347	−6
Meningitis	7.2	50.7	22.7	58.2	6.5	109	137	317		19	28	2.1	332	93	351	−19
Meningitis	7.2	50.1	22.6	63.0	7.0	284	139	320		21	23	2.7	346	99.3	349	−3
Strep. hem. meningitis	7.0	40.4	18.2	67.2	7.52	212	134	309		18	19	3.5	332	93	359	−27
Mastoiditis	7.5	60.0	27	21	2.3						20	1.05	412	116	370	+42
"Normal"	7.52	63.2	28.5	9	1.0		140	324	9	17	14	0.64	442	124	365	+77
Pneumonia	7.49	62.1	28	18	2.0		141.5	329	7.9	15	44	0.41	403	113	364	+39

sults confirm, though not entirely quantitatively. Between the plasma in the circulation and the body fluids outside the circulation a Donnan equilibrium must exist. The analyses of Loeb, Atchley and Palmer[5] have shown that in the case of edema fluid, the membranes separating the circulating blood from the transudate behave differently from the membranes separating the erythrocytes from the plasma in that Na^+ and K^+ are readily interchanged, and under normal conditions proteins do not pass through. The corpuscular membrane is impermeable to Na^+ and K as well as protein. They found that the Donnan equilibrium can be applied to measure the distribution of the ionic constituents between plasma and spinal fluid, as well as between corpuscles and plasma. Hamilton[6] has determined the distribution ratios between blood plasma and spinal fluid. He found that the spinal fluid maintained a Donnan equilibrium with the blood plasma less regularly than edema fluid, but that the same general laws could be applied. Eighteen of his patients were epileptics, in whom there was an increase of lactic acid in the spinal fluid. He did not, however, make allowance for this factor which might have been one of the disturbing factors to which he refers. From the fundamental postulates of the Donnan equilibrium, we have

$$(Na^+)_s = (Cl^-)_s + (P_1^-)_s$$
$$(Na^+)_{sp} = (Cl^-)_{sp}$$

where P_1 is the concentration of the protein in the serum bound as negative ion to base, and Na^+_s and Na^+_{sp} and Cl^-_s and Cl^-_{sp} are in serum and spinal fluid respectively.

The normal spinal fluid is practically protein free so that in accordance with the Donnan equilibrium we have

$$r = \frac{(Na^+)_s}{(Na^+)_{sp}} = \frac{(Cl^-)_{sp}}{(Cl^-)_s + (P_1)_s}$$

Cl^-_{sp} would have to be greater than Cl^-_s. This is normally found to be the case. In cases of meningitis where we have an increased protein content of the spinal fluid, this would tend, in accord with the ratios expressed above, to lessen the chloride content of the spinal fluid and more nearly approximate it to that of the serum. This influence cannot be the paramount one, even in the severest cases of meningitis, because the maximum amount of protein ever found is less than one milliequivalent per 100 c.c. But another factor plays a rôle here

$$\frac{(Cl)^-_s}{(Cl)^-_{sp}} = \frac{(HCO_3)^-_s}{(HCO_3)^-_{sp}} = r$$

When the lactic acid, which is a stronger acid than carbonic enters, the result is a driving off of CO_2 from the spinal fluid so that the ratio given above does not hold momentarily and Cl^- migrates back into the bloom stream in an effort to restore the normal distribution ratio. Hence, in all cases of meningitis, there is a significant lowering of the spinal fluid chloride and a decrease in the gradient between spinal fluid chloride and plasma chloride. This point is of diagnostic value.

If we were to add to 1 liter of perfectly pure water of P_H 7.0, 1 c.c. of 0.01 N HCl the resulting solution would be about P_H 5.0 and very toxic to certain bacteria. If, on the other hand, we were to add the same amount of acid to 1 liter of a standard beef infusion of P_H 7.0, the resulting change of P_H would be scarcely appreciable. This power of resistance to change in P_H with added acid is known as the buffer power of the solution. If acid produced by the catabolic processes of the body is added to blood, there is extremely little change in P_H because of the action of the hemoglobin and the serum proteins which, acting as buffers, stabilize the reaction. These are missing from the spinal fluid, which then becomes dependent on the carbonates for any real buffer power. It can be seen that, since the real stimulus of the respiratory center is due to changes in the P_H content of the blood, and a change in P_H of 0.1 has an enormous effect on the respiratory center, that the need for stability of reaction of the actual fluid, which probably bathes the respiratory center, is very great. The carbonates are the only possible buffer. It has been shown that the maximum buffer effect of a weak acid and its salt is attained when this acid and salt are present in equimolecular quantities. In blood the carbonates play a subsidiary rôle in keeping the hydrogen ion content stable. There is normally present in the blood about 20 equivalents of $NaHCO_3$ but only 1.2 equivalents of carbonic acid. Hence the carbonate buffer value must be very low. In cases of meningitis, there are about 8 equivalents of carbonic acid in spinal fluid released by the lactic acid. Hence the ratio instead of 20/1.2 becomes 12/8.5 which is a much nearer approach to maximum buffer action. The analogy to the low bicarbonate content of the blood and somewhat depressed P_H in the terminal stages of diabetic coma due to the accumulation of β oxybutyric acid is at once apparent. However, there is a fundamental difference, because the accumulation of the CO_2 at the expense of the plasma bicarbonate results instantly in such a powerful stimulus for the respiratory center that the breathing rate is accelerated and the blood passes through the lungs so much more often that the increased oxygenation results in a CO_2 plasma content that is even lower than normal and the buffer action is depressed. With the spinal fluid there is no such passage through an aerating mechanism and the buffer power of the spinal fluid is increased by the accumulation of carbonic acid. The lactic acid itself cannot function as a buffer since its maximum buffer action is only exerted around P_H 4.0 and at the P_H of the spinal fluid its buffer action is practically 0.

Summary.—In the spinal fluid of patients suffering from meningitis there is a decrease in the spinal fluid chloride content and a decrease in the spinal fluid chloride, blood plasma chloride ratio which can be predicted from the postulates of the Donnan equilibrium. There is an increase in the lactic acid content of the spinal fluid and a lowering of the spinal fluid carbonate, blood plasma carbonate ratio. This leads secondarily to an increase in the buffer power of the spinal fluid carbonates and helps maintain the spinal fluid nearer its physiologic P_H.

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STUDIES ON ANTIPEPSIN WITH A NEW QUANTITATIVE METHOD FOR ITS DETERMINATION*

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THE predilection of chronic ulcerations of the gastrointestinal tract for the stomach and duodenum suggests that an important factor to be considered in the etiology of peptic ulcer is some peculiarity in the physiology of the stomach. This organ is the only part of the alimentary canal which secretes hydrochloric acid and pepsin. It is doubtful whether a high free hydrochloric acid content is of much importance in the etiology of peptic ulcer, for there are too many patients suffering with peptic ulcer whose gastric contents show no free hydrochloric acid. If peptic ulcer is a local digestive process of the gastric wall, there must be some explanation for the occurrence of this local digestion in the stomachs of some people and not in others. One theory states that certain people are deficient in some inhibitory agent which normally prevents the pepsin in the stomach from digesting the gastric wall. The blood, in flowing through the tissues of the stomach, may there deposit or excrete this protective antipeptic substance.

Weinland[1] extracted a substance from Ascaris worms which would neutralize and inhibit the activity of pepsin, thus explaining the passage of parasitic worms through the stomachs of their hosts unharmed. Earlier workers, including Sachs,[2] Schwarz,[3] Beitzke and Newberg,[4] and Katzenstein,[5] reported positive results in the attempted demonstration of antiferments in the blood sera of animals. Later contributors to the subject, as Burge,[6] Bayliss,[7] Orator,[8] Keiser,[9] and Hilarowicz and Mozolowski,[10] maintain that no one has yet proved the existence of antipepsin.

This study was undertaken to work out a method which would be satisfactory in the detection and possibly in the quantitative estimation of antipepsin, and to ascertain if such a substance could be demonstrated in the sera and tissues of normal animals and animals subjected to a course of injections with pepsin.

METHODS

1. The principle of the method finally evolved is the peptic digestion of albumin. Ten small tubes are set up in a rack, and to each are added fresh preparations of 0.7 per cent solution of dried egg albumen, 0.0005 per cent stock solution of Merck's C. P. pepsin in 10 per cent glycerine, 2.5 per cent hydrocloric acid, 0.1 c.c. of a 0.04 per cent solution of serum, and water, in the quantities indicated in

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Table I. The solubility of preparations of egg albumen vary. In this procedure, the albumin solution must contain 0.7 per cent protein by chemical analysis. The control set up is exactly like the other, except that instead of containing the serum to be tested, it has either the serum of normal, noninjected animals, or a quantity of additional albumin solution whose protein content is exactly the same as that of the serum being tested.

The system is placed in an incubator at 37.5° C. After one hour has elapsed, the tubes are removed, and to the contents of each is added one cubic centimeter of 4.5 per cent trichloracetic acid. The undigested protein is immediately precipitated as a white cloud, the intensity of which varies inversely with the degree of peptic digestion of the protein.

The measurement of peptic digestion may be done by either visual or chemical methods. In the former, the tubes are held up to the light, and the cloudiness in the control is compared to that of the tube which contains the serum in question. A nephelometer would undoubtedly improve the accuracy of this method. Chemical estimations are most accurate, and are made by measuring the amount of nonprotein nitrogen in each tube by an adaptation of the Folin-Farmer[11] method for total nitrogen determinations in urine.

2. In order to determine the antipeptic effect residing in the tissues of the animals which had received injections of pepsin, two methods were devised which are adaptations of the above procedure. The principle of one of them is the direct digestion of the tissue in question by pepsin. Test tubes containing water, 2.5 per cent hydrochloric acid, and amounts of pepsin to make up a final concentration of this substance varying from 0.0002 per cent to 0.0001 per cent, are set up. One gram each of fresh liver, muscle, duodenum, and stomach mucosa from 4 pepsin-injected animals and two non-injected animals (controls) is placed in the test tubes, each tissue being tried in three different concentrations of pepsin. After an hour at 37.5° C., 4.5 per cent trichloracetic acid is added, and nonprotein nitrogen determinations made on the filtrates of the contents of each tube.

3. The third procedure is based upon the effect of glycerine tissue extracts on peptic digestion of albumin. The set-up is similar to that given in Table I. In-

TABLE I

	1	2	3	4	5*	6	7	8	9	10
Albumin	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
HCl	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Serum	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Pepsin	2.	1.2	0.6	0.24	0.12	1.	0.75	0.3	0.15	0.1
HOH	0.15	0.95	1.55	1.91	2.03	1.15	1.4	1.85	2.	2.05
Total	3 c.c.	3 c.c.	3 c.c.	3 c.c.	3 c.c.	3 c.c.	3 c.c.	3 c.c.	3 c.c.	3 c.c.

*In tubes 5-10 a 0.00004 per cent pepsin solution is used.

stead of serum, however, 0.2 c.c. of extracts obtained by extracting 5 gm. of liver, muscle, duodenum, and stomach mucosa for twenty-four hours at room temperature with 75 c.c. of 65 per cent glycerine, are used. Nonprotein nitrogen determinations are made on the filtrates of the contents of each tube.

RESULTS

1. To prove the sensitivity of our method for the detection of antipepsin, it was necessary to test it on some substance known to contain antipepsin. Accordingly 3 gm. of dry, powdered Ascaris was extracted with 50 c.c. of 50 per cent glycerine for eighteen hours, filtered, and the filtrate diluted one to four. The procedure described under methods (1) was carried out, using 0.2 c.c. of this diluted material instead of the serum. Results are indicated in Chart 1, and demonstrate that when the pepsin concentration falls below 0.00004 per cent, there is definite inhibition of peptic activity in the tubes where the Ascaris extract was used.

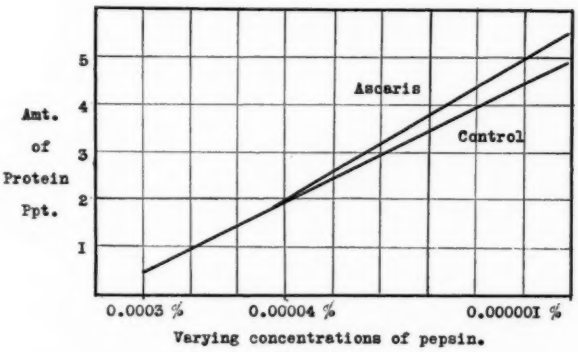
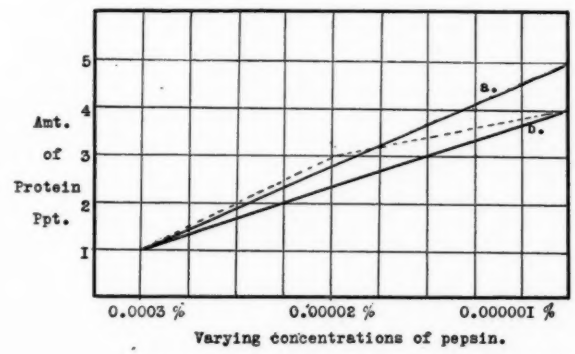


Chart 1.



* Dotted line represents curve before injections. (control curve identical).
a. Serum of animal after injections.
b. Serum of control animal to a.

Chart 2.

2. The sera of twelve normal, healthy, rabbits were tested by the procedure described under methods (1). Using both visual and chemical methods of measurement, it was found that half the animals showed an antipeptic activity of the blood serum and the other half did not.

3. Two animals which showed no antipeptic activity of the blood sera were given intravenous injections of 0.2 gm. of pepsin (Merck, C. P.) every five days for one hundred and fifty days. After a few weeks, the sera of both showed antipeptic activity. Chart 2 gives the results of the serum tests on one animal before and

after injections. Marked antipeptic activity occurs when the pepsin concentration is less than 0.0003 per cent. Seventy-seven per cent of nonprotein nitrogen determinations confirmed the results.

Five animals were injected intravenously for one hundred and fifty days every five days, beginning with 0.01 gm. and increasing the dosage by 0.025 gm. each time. Three of these animals had shown no antipeptic activity of the blood sera before injections, and developed it after several injections. Seventy-six per cent of the chemical determinations confirmed these results.

4. Eighty-three per cent of the nonprotein nitrogen determinations obtained by using the method of direct digestion of tissue by pepsin for estimating how much antipepsin may be detected in the tissues of animals, demonstrated, in 2 rabbits, that the livers of the pepsin injected rabbits were antipeptic as compared with the livers of noninjected animals. With the other tissues, duodenum, muscle, and stomach mucosa, only 60 per cent of the determinations showed antipeptic activity.

5. The results obtained by finding the effect of glycerine extracts of tissues of 4 animals on peptic digestion of albumin, showed that 83 per cent of the nonprotein nitrogen determinations demonstrated a high antipeptic activity of the muscle extracts, and a low one (60 per cent) with the other tissue extracts.

DISCUSSION

In the methods described, the P_H is well controlled. This is important, for changes in the reaction of a medium will definitely alter the activity of the enzyme contained. In fact, Bayliss[7] and Popper[12] concluded that all positive reports on antipepsin are due to the unsatisfactory control of the hydrogen ion concentration. In the set-up described, although the reaction might possibly vary slightly from week to week, it could not vary between the contents of any given control tube and the contents of the tube which is being tested. The presence of a strong mineral acid (HCl) tends to keep the P_H constant. No salt is introduced into the solutions used, thus ruling out the possibility that it is responsible for any inhibition of peptic activity, as has been asserted by Hamburger.[13]

The fact that the same amount of protein is present in the solutions of the control and test tubes prevents the supposition that an antipeptic effect is due to the absorption of pepsin by the protein. Trichloracetic acid was chosen as the precipitating agent, because in the concentration used, it will precipitate the protein out of a solution of partially digested protein without carrying down the protein split products.[14]

The results with the ascaris extract prove that the test is sensitive in the estimation of antipepsin. Only one-half the normal, uninjected rabbits showed any antipepsin activity of the blood serum. There may be a variation in antipepsin in different animals, or quite possibly, the substance may be present in such small amounts normally that the test devised is not sufficiently sensitive to detect it. The test is sensitive enough, however, to detect the development of antipeptic activity of the blood stream when a course of injections with pepsin has been given. Six animals showed the presence of antipepsin in the blood stream after intravenous injections of pepsin.

Results seem to point to the liver as being the tissue where most of the antipepsin resides, although the extract of liver was not very antipeptic, due perhaps to the

influence of the new factor, namely, glycerine extraction. The liver being the most active site of antibody formation, this would strengthen the belief that the antipeptic quality detected in the blood stream and tissues of the animal used, is an antibody-like substance. It is certainly, however, not produced in anything like the quantities which antibodies are usually produced. Kolmer[15] believed that there are anti-antiferments which prevent the accumulation of too much antiferment. It is felt that if the methods described were used to determine the antipeptic activity of the blood sera of patients suffering from peptic ulcer, results might be obtained which would have some bearing on the problem of the etiology of peptic ulcer.

CONCLUSIONS

1. A new method is presented for the detection and relative quantitative estimation of antipepsin.
2. Rabbits, injected intravenously with pepsin over a period of time, showed the development of an antipeptic activity in the blood sera.
3. This antipeptic activity is due to a real antibody, namely, antipepsin.
4. The liver is the organ with the largest store of antipepsin.

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THE EFFECT OF SHORT RADIO WAVES ON THE BIOLOGIC ACTIVITIES OF SOME BACTERIAL SPECIES*

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THE discovery that an increase of temperature was produced in substances or animal bodies exposed to short radio waves, has stimulated investigation regarding the possible application of this new knowledge to medical therapy. From the experiments undertaken data have also accrued, which are of more academic interest.

HISTORICAL REVIEW

Schereschewsky's early work on mouse tumors[1, 2] was followed by Hosmer's[3] report on the effect of heating various electrolytes under different conditions. She also observed the resultant tissue changes when rats were heated by exposure to short radio waves. Pariseau[4] observed that egg could be coagulated by the action of the waves acting in very definite sites in the field between the plates. Kahler[5] found that the action on paramoecia was strictly comparable to that produced by ordinary means of heating these animals. MacCreight and McKinley[6] killed mice by exposure to short radio waves. Carpenter and Page[7] reported upon their experiences in exposing human subjects to short radio waves. Later, Carpenter and Boak[8] showed that increased temperature in rabbits produced by short radio waves could prevent experimental syphilis. They, as well as other investigators, observed that the cells of different portions of the body did not respond in a uniform manner. Thus, the temperature of one organ, such as the liver, was greater than that of skeletal muscle. The physiologic and biochemical changes wrought by exposure to a high frequency field have been described by Knudson and Schaible,[9] while morphologic changes were studied by Jacobsen and Hosoi.[10] McLennan and Burton[11] demonstrated the selective heating by this means on pieces of dead tissue.

Szymanowski and Hicks[12] have recently analyzed exhaustively the literature relative to the effects of radiothermy; they have also indicated the results of their own experiments on exposure of thin films of bacterial toxins; they believed that the toxicity of the products was reduced somewhat and that the effect was not due to heat created by the radio waves, since provision had been made to exclude that factor. A further review of the current knowledge regarding this subject has been given by Hemingway and Stenstrom.[21]

Ross,[13] working in this laboratory with the Rous chicken sarcoma, has not been able to confirm Schereschewsky's and Schereschewsky and Andervont's[20] early experience with the mouse tumor. Ross found that exposure of chickens inoculated with the Rous sarcoma did not retard, but rather enhanced the growth of the sarcoma.

EXPERIMENTS

The equipment employed for obtaining the heating effects about to be described consisted of a high frequency heater as shown in Fig. 1, which was the gift of the General Electric Company of Schenectady and the Canadian General Electric Company of Toronto, arranged through the kindness of Dr. Whitney. A wavelength of 30 meters as registered on a wavemeter was consistently used in all the experiments herewith outlined. The plates were set at a distance of eleven inches apart. When the object exposed to the effect of the machine was elevated above the

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floor between the plates, it was supported on a wooden stand at a height of 9¼ inches, as shown in Fig. 1. Both cultures and animals were placed parallel to the field unless otherwise indicated.

EFFECT OF SHORT RADIO WAVES ON BIOLOGIC AND SEROLOGIC CHARACTERISTICS OF BACTERIA

On Pigment Production and Colony Form.—B. pyocyaneus, spread on a plain nutrient agar plate, P_H 7.4, was incubated for eighteen hours. The cover was removed, the plate secured on the wooden stand in a vertical position at right angles to the plane of the field between the plates. Exposure was continued for one and one-half hours. Colonies were transferred from this plate to a fresh plate. After incubation no diminution in the power to produce the characteristic pigments of B. pyocyaneus was noted, as the culture possessed the same intensity of greenish yellow color as the original culture.

An eighteen-hour nutrient broth culture of B. prodigiosus was placed in a sausage-shaped pyrex T-tube, such as McLennan and Burton[11] used, and heated for fifteen minutes in the machine. The temperature taken at the end of that time was 62° C., an increase of 30° C. having taken place in the fifteen minutes. A bacteriologic loopful of the culture was streaked on a plain nutrient agar plate. Abundance of pigment was shown after the plate was incubated, and the "smooth" colony form had not changed.

B. prodigiosus was also heated in a pyrex T-tube surrounded by a pyrex jacket. In the outer chamber was an electrolyte consisting of sodium chloride at a concentration of 0.0045 gm. molecules per liter as shown by McLennan[14] and Burton to give an optimum effect. The temperature rose 32° C. in a fifteen-minute exposure. Again no change was noted in the effect produced in the culture either as to pigment production or colony form.

On Biologic Characteristics.—Salmonella muriotitis was spread on a nutrient agar plate and treated as the pyocyaneus and prodigiosus cultures for one and one-half hours. Colonies were transferred from this plate to a fresh agar plate and to agar slopes. The "smooth" colony form was retained. Indol and motility tests, the gas-producing power in carbohydrate medium, the action on litmus milk, the ability to reduce lead acetate and to produce acid in medium containing various carbohydrates were then examined. The cultures selected from the plate exposed to the action of the 30 M radio waves had neither lost nor gained characteristics which differed in any respect from those possessed by the original culture.

S. muriotitis was not killed when heated for twenty-five minutes in the machine, the final temperature reached at the end of that time being 80° C.

On the Antigenic Factors of Bacilli.—A broth culture of S. muriotitis was subjected to a 30 meter wave for nine hours, at the end of which exposure the temperature of the culture was 41° C. The culture was living. The suspension was centrifuged, resuspended in 0.85 per cent sodium chloride, P_H 7.0, and employed as the antigen for the production of an agglutinating serum in a rabbit (serum A). The results obtained by using this serum are compared with those which were given by an agglutinating serum (serum B), the antigen for which was prepared by heating in a water-bath at 56° C. for one hour prior to injection (Table I). The sera were tested with fresh unheated eighteen-hour suspensions of S. muriotitis, with a sus-

pension heated at 60° C. for one hour in a water-bath, and with a suspension heated for one hour at 100° C.

From Table I it is to be noted that there is no essential difference in the results. Serum A apparently had a higher titer than Serum B. Similar agglutinins[15, 16] were present in both sera. Exposure to heat produced by short radio waves ap-

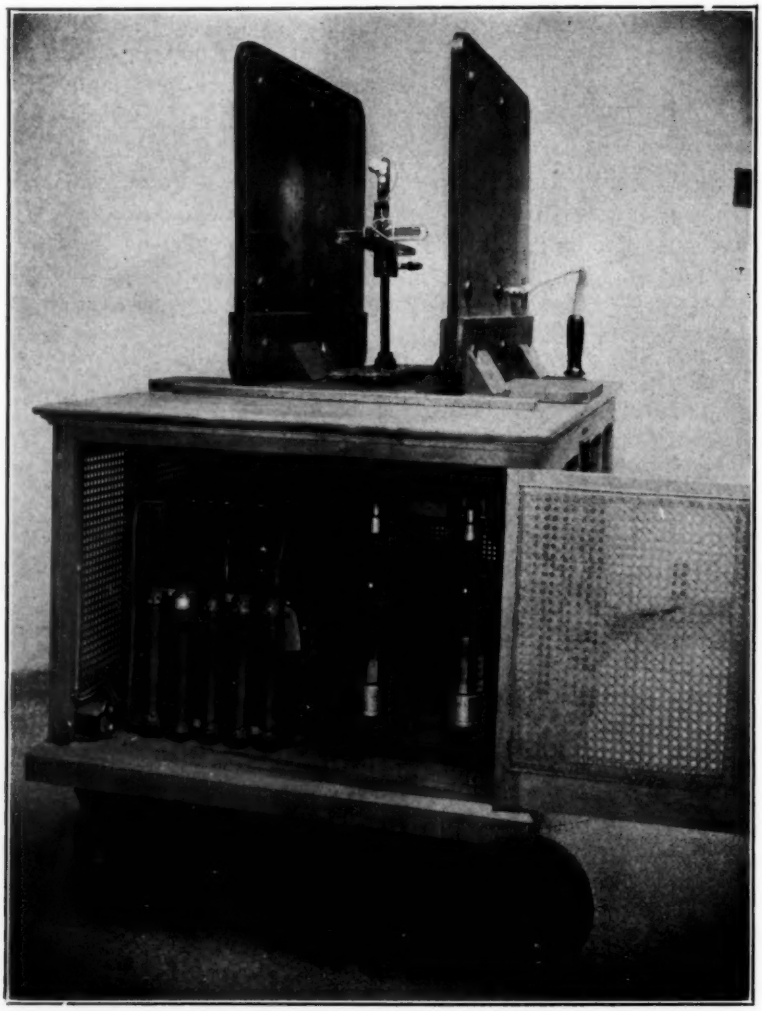

Fig. 1.

parently has not influenced the agglutinin antigenic factors of bacteria as far as these experiments show.

PNEUMOCOCCUS EXPERIMENTS

An eighteen-hour broth culture of a strain of Pneumococcus, Type II, which was fully virulent, having been mouse-passed just before use, was employed in these experiments. A temperature of 41° C. was attained by heating one portion of the culture in a water-bath for twenty-five minutes (A). A second portion was

TABLE I

AGGLUTINATION TESTS USING SERA PREPARED WITH ANTIGEN HEATED BY 30 M RADIO WAVES, AND WATER-BATH HEATED ANTIGEN*

SERUM	CULTURE	SERUM DILUTIONS								
		40	80	160	320	640	1280	2560	5120	10240
Serum A Antigen Exposed to 30 M Radio Waves for 9 hours.	Unheated	4	4	4	4	4	4	4	2	0
	Heated at 60° C.	4	4	4	4	4	4	2	1	0
	Heated at 100° C.	3	3	2	2	2	1	0	0	0
Serum B Antigen Heated in Water-bath at 56° for one hour.	Unheated	4	4	4	4	2	1	0	0	0
	Heated at 60° C.	4	4	4	2	1	0	0	0	0
	Heated at 100° C.	2	1	0	0	0	0	0	0	0

*4, Complete agglutination.
3, Almost complete agglutination.
2, Partial agglutination.
1, Very little agglutination.
0, No agglutination.

heated in the machine for twenty-eight minutes, the final temperature being 41° C. (B). Each of five mice was injected intraperitoneally with 0.1 c.c. of Culture A; five other mice received 0.1 c.c. each of Culture B. A third lot of five mice received a similar injection of unheated culture and were then exposed to 30 M waves for five minutes (C). A control group of five mice (D) received a similar injection of unheated culture. No temperatures were taken.

All animals which received Culture B and D died (Table II, experiment I); two belonging to Group C died, the remainder surviving until disposed of ten days later. None of Group A died. Either the virulence was much attenuated by the water-bath heating, or the culture was not living. Cultures identical with the injected culture were isolated from the heart's blood of the autopsied mice as identified by bile solubility.

This experiment was repeated (Table II, Exper. II). The water-bath heating of the culture was continued for twenty minutes and the final temperature was 40° C. The culture heated in the machine was exposed for 30 minutes, but the final

TABLE II

PNEUMOCOCCUS EXPERIMENTS

EXER. NO.	CULTURE WATER-BATH HEATED (A)		CULTURE 30 M WAVE HEATED (B)		UNHEATED CULTURE MICE EXPOSED TO 30 M WAVE (C)		UNHEATED CULTURE CONTROLS (D)	
	NO. MICE	NO. DEAD	NO. MICE	NO. DEAD	NO. MICE	NO. DEAD	NO. MICE	NO. DEAD
I	5	0	5	5	5	2	5	5
II	5	5	5	5	5	5	5	5
III	0	0	0	0	5	3	5	5
IV	3	3	3	3	0	0	3	3

temperature was 34° C. All animals in the four divisions died within forty-eight hours.

Ten mice injected with pneumococcus were divided into two lots of five each; one lot was exposed to 30 M waves for two minutes; the control lot was not so treated (Table II, Exper. III). Of the test lot, one mouse died immediately after injection, and a second just after having been exposed to the action of the machine. The three which remained and the five in the control group were dead in forty-eight hours. Pneumococci were isolated from the heart's blood.

A fourth experiment of a similar nature was carried out with nine mice divided into three lots of three each. One lot was injected with culture heated in water-bath to a temperature of 42° C.; the second lot, culture heated in the machine for one hour to a temperature of 41° C.; the third with unheated culture. All animals died, and again pneumococci were isolated from the heart's blood.

Pneumococcus did not lose its virulence for mice when exposed to 30 M waves for the length of time indicated, which caused an increase in the temperature of the culture to the degree noted. Mice which have received a lethal dose of pneumococcus and were immediately exposed to 30 M radio waves, developed their infection and died as rapidly as did the control animals which received unheated culture and were not exposed to radio waves.

Kapeloff and Holden,[17] carrying out analogous experiments with herpes virus, found that radiothermy had no effect on the virulence of the virus.

VITAMIN DEFICIENT ANIMALS

Experiments were carried out using rats which were on a vitamin D deficient diet, with a control group receiving vitamin D in a diet which included 12½ per cent vitamin D bread. A small amount, 0.08 c.c., of an eighteen-hour broth culture of Salmonella muriotitis was fed by mouth. This volume was sufficient to cause death in such animals fed in this manner, as determined by Robertson and Ross[18, 19] in their experiments on resistance to infection in rats on a vitamin deficient diet. In the first experiment the animals were given an exposure of about five minutes three days after being fed the culture. The infection is established by that time, as ascertained by Robertson and Ross.[19] In the second experiment the animals were not exposed until six days after the culture was fed, when the duration of exposure was from three to eight minutes.

While the number of animals in the two experiments (Table III) was small, it is seen that more vitamin D deficient rats died from the infection after exposure to the effect of short radio waves than did those not exposed, or did the animals whose diet was not deficient in this respect.

An eighteen-hour broth culture of S. muriotitis was heated in a water-bath for two hours at 40° C., which temperature failed to kill the culture. A similar culture was heated to 35° C. by 30 M radio waves. One-tenth of a cubic centimeter of these cultures was fed to rats suffering with vitamin B deficiency. Of the five animals fed the radio-wave heated culture, all died, while none of the five rats fed the water-bath heated culture died. The 40° C. temperature of the latter culture may have attenuated its virulence more than the 35° C. temperature obtained by radiothermy.

Other experiments were carried out with young vitamin B deficient rats in which the culture was fed, after which the animals were exposed to the 30 M wave.

TABLE III

EFFECT OF 30 M RADIO WAVES ON INFECTION IN VITAMIN D DEFICIENT RATS

	D DEFICIENT				CONTROLS (+ VITAMIN D)			
	EXPOSED		NOT EXPOSED		EXPOSED		NOT EXPOSED	
	NO. RATS	NO. DEAD	NO. RATS	NO. DEAD	NO. RATS	NO. DEAD	NO. RATS	NO. DEAD
I	2	2	2	1	2	1	2	*2
II	3	2	2	0	3	**1	2	1
	5	4	4	1	5	2	4	3

*Died in 72 hours. No S. muriotitis isolated.
**Died while being rayed. No S. muriotitis isolated.

The controls in this group were supplied the vitamin in the form of yeast. The animals were fed by mouth 0.08 c.c. of an eighteen-hour broth culture of S. muriotitis by the same method as was used with the vitamin B deficient rats. The rats in both test and control group were then given one exposure to the 30 meter radio waves for about eight minutes. All the vitamin deficient animals were dead within seventy-two hours, while a number of the controls survived. Of the controls which died, death did not occur before the end of five days, and was delayed in one animal for thirteen days. S. muriotitis was recovered from the heart blood.

No unusual phenomena were produced by heating the bacterial culture by radio waves, which could not also be the result of more ordinary methods of heating. If this were not so, long continued exposure of the bacteria which resulted in a sub-lethal temperature would have ample opportunity to bring about such changes.

The exposure of animals which had been receiving diets deficient in vitamin B, as of those whose diet had been deficient in vitamin D, does not curtail to any significant extent the acute infection produced in such animals by the means described.

Experiments undertaken with greater numbers of animals under conditions other than those outlined in this report might produce different results. These experiments indicate, however, that acute infections are not aborted by radiothermy.

SUMMARY

1. Heat generated by short radio waves had no effect on the colony form, pigment production, the biologic or some agglutinin antigenic properties of bacteria.

2. The virulence of bacterial species was not attenuated by radiothermy.

3. Short radio waves did not influence acute infections (such as pneumococcus may produce) to which mice are susceptible. Neither did exposure to radiothermy curtail a per os infection in vitamin deficient white rats.

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STUDIES ON THE NORMAL BLOOD. III. THE GUINEA PIG*

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THERE is available little literature on the normal blood pictures of the animals which are commonly used in laboratory investigations. Experimentation often requires a knowledge of the normal hemoglobin percentage, the normal number of red cells and the normal number of white cells and their distribution. We have been interested here during the last three years in making such studies on the animals which are more commonly used in the laboratory. I have[1] published a detailed account of a study on the blood of the normal dog.

It was the purpose of the work herein reported to determine in normal animals of both sexes and of different ages:

a. The hemoglobin percentage
b. The erythrocyte number
c. The total and differential white blood cell counts

All animals were kept in the laboratory for a period of two weeks before beginning the experiments. The diet consisted of hay, oats, cabbage, lettuce, carrots, and grass. The animals appeared to be normal in every respect and exhibited no nervous reactions during the obtaining of blood for the counts.

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The usual clinical methods were employed throughout the work. For making the leucocyte count the Leitz hemocytometer with Neubauer ruling was used. The dilution pipette afforded a 1 to 20 or 1 to 10 dilution. Turk's solution was the diluting medium. The blood was drawn to 0.5 mark and the diluting solution immediately drawn up to 11 mark (1 to 20 dilution). In counting, the cells in four large squares of sixteen small squares each were counted and their sum multiplied by fifty.

The Leitz hemocytometer with Neubauer ruling was also used in the erythrocyte determinations. The diluting pipette afforded a dilution of 1 to 200 or 1 to 100. Hayem's solution was the diluting medium. The blood was drawn up to the 0.5 mark and immediately diluted to the 101 mark (1 to 200 dilution). The cells in five squares of sixteen smaller squares each (80 small squares) were counted and the sum multiplied by 10,000.

The cover glass method was employed in making blood smears. The smear was dried in air and stained with Wright's stain for one minute. Distilled water was placed on the smear for four minutes, after which the preparation was rinsed under tap water and allowed to dry. The cells were studied under oil immersion.

In the differential counts, all neutrophils, basophils, and eosinophils were grouped as polymorphonuclears. There were some differences among the various polymorphonuclears, but the granules in no case stood out as they do in the human basophils and eosinophils. The unquestionable neutrophils had a heavily stained irregular nucleus with a pale or almost colorless cytoplasm, while the cytoplasm of the polymorphonuclears was somewhat granular, but the granules were too small, and the color too indefinite to class them as eosinophils or basophils.

GUINEA PIG 1.—MALE, THIRTY WEEKS OLD

	HEMOGLOBIN PER CENT	TOTAL RED	TOTAL WHITE	LYMPHOCYTES P.M.N. PER CENT	LYMPHOCYTES LARGE-SMALL PER CENT	LYMPHOCYTES TRANS. PER CENT
First week	79	5,170,000	13,600	64	35	1
Second week	80	5,520,000	13,400	52	47	1
Third week	80	4,930,000	13,900	58	42	0
Fourth week	78	4,970,000	14,000	60	38	2
Fifth week	79	4,830,000	15,800	57	39	4
Sixth week	80	5,140,000	14,600	54	44	2
Average	79	5,093,000	14,200	57	41	2

GUINEA PIG 2.—FEMALE, THIRTY WEEKS OLD

	HEMOGLOBIN PER CENT	TOTAL RED	TOTAL WHITE	LYMPHOCYTES P.M.N. PER CENT	LYMPHOCYTES LARGE-SMALL PER CENT	LYMPHOCYTES TRANS. PER CENT
First week	77	5,300,000	14,600	63	35	2
Second week	78	5,380,000	14,000	63	37	0
Third week	76	5,141,000	13,400	64	35	1
Fourth week	77	5,090,000	14,400	67	30	3
Fifth week	78	5,140,000	14,400	65	33	2
Sixth week	77	5,150,000	14,000	56	44	0
Average	77	5,200,000	14,100	63	35	1

GUINEA PIG 3.—MALE, THIRTEEN WEEKS OLD

	HEMOGLOBIN PER CENT	TOTAL RED	TOTAL WHITE	LYMPHOCYTES P.M.N. PER CENT	LYMPHOCYTES LARGE-SMALL PER CENT	TRANS. PER CENT
First week	73	4,810,000	14,600	53	47	0
Second week	75	4,030,000	15,200	64	36	0
Third week	74	4,260,000	16,000	59	40	1
Fourth week	74	4,330,000	14,600	52	46	2
Fifth week	75	4,830,000	16,400	55	42	3
Sixth week	75	4,940,000	15,400	66	34	0
Average	74	4,533,000	15,270	58	41	1

GUINEA PIG 4.—FEMALE, THIRTEEN WEEKS OLD

	HEMOGLOBIN PER CENT	TOTAL RED	TOTAL WHITE	LYMPHOCYTES P.M.N. PER CENT	LYMPHOCYTES LARGE-SMALL PER CENT	TRANS. PER CENT
First week	74	4,280,000	14,800	64	36	0
Second week	74	4,200,000	15,400	61	36	0
Third week	75	4,650,000	14,800	67	32	1
Fourth week	74	4,200,000	14,000	56	41	3
Fifth week	75	4,770,000	15,000	69	31	0
Sixth week	73	4,540,000	14,400	59	39	2
Average	74	4,440,000	14,700	63	36	1

SUMMARY

	HEMOGLOBIN PER CENT	TOTAL RED	TOTAL WHITE	LYMPHOCYTES P.M.N. PER CENT	LYMPHOCYTES LARGE-SMALL PER CENT	TRANS. PER CENT
Guinea Pig 1	79	5,093,000	14,200	57	41	2
Guinea Pig 2	77	5,200,000	14,100	63	35	1
Guinea Pig 3	74	4,533,000	15,270	58	41	1
Guinea Pig 4	74	4,440,000	14,700	63	36	1
Average	76	4,816,500	14,560	60	38	1

For the hemoglobin, the Sahli method was used. This consists in filling the estimation tube to the 10 mark with tenth-normal hydrochloric acid and carefully expelling the blood, which has been drawn up to the 20 mm. mark in the hemoglobin pipette, below the surface of the acid. The blood is allowed to hemolyze for two minutes and then diluted with distilled water until the color matches the standard color rods. The percentage is then read directly. Two counts were made on all experiments, the results checked to within experimental error. White cells varied not more than 200 to 250, and red cells 50,000 to 100,000. A mean of the two counts was recorded.

A survey of the many blood counts above shows:

a. The total white counts of ages from thirteen weeks to thirty weeks average 14,560.

b. There are no constant differences in the total white counts of the guinea

pigs of ages from thirteen to thirty weeks or between males and females of these ages.

c. There is an average red cell count of 4,816,500 and the ranges are similar in both males and females of the ages studied.

d. The hemoglobin percentage averages 76.

e. The lymphocytes, large and small, were difficult to separate. They are, therefore, grouped together.

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LABORATORY METHODS

THE COLORIMETRIC DETERMINATION OF PLASMA PROTEINS*

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IF AN alkaline solution of the blood protein is heated for a short time, the chromogenic value, when treated with Folin-phenol reagent, is markedly intensified. Thus serum-globulin gives half again as much color when so treated and serum-albumin twice as much. A maximum chromogenic effect is produced by one-half hour heating in a water-bath, using 10 per cent sodium hydroxide as the protein solvent.

This procedure is introduced into the following adaptation of the colorimetric method of Wu,[1] Wu and Ling,[2] and the late modification of Greenberg.[3] Further simplifications of the technic of the colorimetric protein method have been introduced and a rapid and more accurate colorimetric procedure for the determination of blood proteins is offered.

Greenberg, in his modification, used sodium sulphate according to the Howe separation of protein.[4] The necessity of keeping the sodium sulphate at 37° to keep it in solution, and the difficulty of filtration, besides the long time necessary for the complete precipitation of the globulin, led us to discard the Howe procedure and use the precipitation with ammonium sulphate. Robertson[5] reviewed the methods for the separation of protein and concluded that the ammonium sulphate precipitation was the most satisfactory. Wu, and Wu and Ling, as well as Cullen and Van Slyke[6] also found this a satisfactory separation.

Wu had noted in his colorimetric determination of protein that if the protein precipitate was allowed to stand with sodium hydroxide for a short time, or with sodium carbonate for a long time, that the chromogenic value was increased, but he made no attempt to incorporate this into his method.

We found that by heating for a half hour, the chromogenic value not only is constant and maximum, but the tyrosine equivalents for the plasma proteins are roughly the same. This permits the determination of the total proteins of plasma and spinal fluid with sufficient clinical accuracy irrespective of the protein partition.

The development of color with the Folin-phenol reagent[7] was considered satisfactory. This reagent gives some precipitate; this, however, has no effect on the color, and settles so rapidly that it does not interfere with the colorimetric reading. We did not find that modified reagent by Folin and Ciocalteau[8] gives an appreci-

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ably deeper color with the alkaline protein than did the original reagent as stated by Greenberg.

METHOD

Procedure.—To 1 c.c. of plasma in a centrifuge tube, add 2 c.c. of water, and 1 c.c. of saturated ammonium sulphate (56 gm. per 100 c.c.). Mix and centrifuge after a few minutes to separate the fibrinogen. Pour off the supernatant fluid containing the albumin and globulin, and reserve the precipitate of fibrinogen.

One cubic centimeter of the supernatant fluid is pipetted into a 15 c.c. centrifuge tube, and 0.5 c.c. of water and 1 c.c. of saturated ammonium sulphate added. This brings the solution to half saturation. Let stand for twelve hours at room temperature, or at 40° for fifteen minutes. Centrifuge and pour off the supernatant fluid for the albumin determination. Dissolve the globulin precipitate in 5 c.c. water.

Pipette 2 c.c. of reserve fluid containing the albumin into a centrifuge tube, and add 3 c.c. of water. Then add 1 c.c. of 20 per cent trichloracetic acid to both the globulin and albumin solutions. Centrifuge and pour off the supernatant liquid. Dissolve the precipitates of albumin, globulin, and fibrinogen in 0.5 c.c. of 10 per cent sodium hydroxide and heat in boiling water-bath for one-half hour. Add 7.5 c.c. water to all the tubes.

To 2 c.c. of 20 mg. per cent tyrosine standard solution (in N/10 HCl), in a centrifuge tube, add 6 c.c. water. To each of the proteins and to the standard, add 1 c.c. Folin-phenol reagent[7] and 3 c.c. of saturated sodium carbonate and mix by inversion. Read after thirty minutes in a colorimeter, setting the standard at 10 mm.

Calculation.—The tyrosine equivalents were found to be:

1 mg. tyrosine = 12.9 mg. albumin
1 mg. tyrosine = 13.1 mg. globulin
1 mg. tyrosine = 13.0 mg. fibrinogen

Albumin.—

$$\frac{10}{R} \times \frac{0.4}{0.2} \times 100 \times 12.9 = \text{mg. albumin per 100 c.c.}$$

$$\text{or } \frac{25.8}{R} = \text{per cent albumin.}$$

Globulin.—

$$\frac{10}{R} \times \frac{0.4}{0.25} \times 100 \times 13.1 = \text{mg. globulin per 100 c.c.}$$

$$\text{or } \frac{21.0}{R} = \text{per cent globulin.}$$

Fibrinogen.—

$$\frac{10}{R} \times \frac{0.4}{1.0} \times 100 \times 13.0 = \text{mg. fibrinogen per 100 c.c.}$$

$$\text{or } \frac{5.2}{R} = \text{per cent fibrinogen.}$$

TOTAL PLASMA PROTEIN

Due to the close agreement of the tyrosine equivalents, it is possible to determine total protein by using 13 as the tyrosine equivalent.

METHOD

Procedure.—Plasma is diluted 1:10 with saline and 1 c.c. is transferred to a 15 c.c. centrifuge tube and 4 c.c. of water and 1 c.c. of trichloracetic acid are added. The precipitate is centrifuged out and the supernatant fluid poured off. The precipitate is dissolved in 0.5 c.c. of 10 per

cent sodium hydroxide and heated in a water-bath for a half hour. The color is then developed as given previously for the fractions.

Standard.—The same standard is used as is used for the fractions.

Calculation.—

$$\frac{10}{R} \times \frac{0.4}{0.1} \times 100 \times 13.0 = \text{mg. protein per 100 c.c.}$$

$$\text{or } \frac{52.0}{R} = \text{per cent protein.}$$

PROTEIN IN SPINAL FLUID

METHOD

Procedure.—Two cubic centimeters of spinal fluid is measured into a 15 c.c. centrifuge tube and 3 c.c. water and 1 c.c. 20 per cent trichloracetic acid is added. The protein precipitate is centrifuged out and the supernatant fluid is poured off. The precipitate is dissolved in 0.25 c.c. sodium hydroxide and heated one-half hour in a water-bath. Then add 3.75 c.c. water, 0.5 c.c. phenol reagent, and 1.5 c.c. saturated sodium carbonate. Mix by inversion and read in a half hour, setting the standard at 10 mm.

Standard.—Two standards should be made up, one containing 1 c.c. 20 mg. per cent tyrosine and 7 c.c. water, and the other 2 c.c. standard and 6 c.c. water. Then add 1 c.c. phenol reagent and 3 c.c. saturated sodium carbonate to each tube.

Calculation.—Standard 1:

$$\frac{10}{R} \times \frac{0.2}{2.0} \times \frac{100}{2.0} \times 13.0 = \text{mg. protein per 100 c.c.}$$

$$\text{or } \frac{650}{R} = \text{mg. protein.}$$

Standard 2:

$$\frac{10}{R} \times \frac{0.4}{2.0} \times \frac{100}{2.0} \times 13.0 = \text{mg. protein per 100 c.c.}$$

$$\text{or } \frac{1300}{R} = \text{mg. protein.}$$

EXPERIMENTAL

In order to determine the tyrosine equivalent of fibrin, 400 mg. fibrin (Kahlbaum) was heated for forty minutes with 50 c.c. of 10 per cent sodium hydroxide and 25 c.c. of water. The solution was made up to 100 c.c. and 1 c.c. taken for the determination. The tyrosine equivalent under the conditions of the method was 12.9.

This value was checked by a nitrogen determination. A macro-Kjeldahl was run on oxalated plasma. To another portion of the plasma a trace of calcium chloride was added, and after twenty minutes, the clot was broken up, and a Kjeldahl run on the serum. The difference in nitrogen values multiplied by 5.91[9, 10] gave the amount of fibrinogen. The equivalent was 13.

The tyrosine equivalents of the albumin and globulin were determined by comparison of the colorimetric determination with the protein values based on nitrogen content of the fractions. Nonprotein nitrogen of the *serum* specimen was determined according to Folin and Wu.[11] The globulin was precipitated by the method of Howe,[4] and Kjeldahl. The nonprotein nitrogen was subtracted from

the total nitrogen of the filtrate from the globulin precipitate. This value and that of the globulin nitrogen were multiplied by 6.29[12] to obtain the amount of these proteins.

A series of determinations was run on normal and patients' serums, and

TABLE I

A COMPARISON OF THE PLASMA PROTEIN VALUES OBTAINED BY THE COLORIMETRIC METHOD AND BY DETERMINATION BASED ON THE NITROGEN CONTENT

SPECIMEN	FRACTION	PER CENT PROTEIN		DEVIATION
		COLORIMETRIC	NITROGEN	
Mixed plasma	albumin	3.38	3.54	−0.16
	globulin	2.64	2.62	+0.02
	total	6.02	6.16	−0.14
Mixed plasma	albumin	3.85	3.75	+0.10
	globulin	2.68	2.62	+0.06
	total	6.53	6.37	+0.16
Nephrosis	albumin	1.99	2.10	−0.11
	globulin	2.40	2.39	+0.01
	total	4.39	4.49	−0.10
Cardiac with edema	albumin	2.90	3.03	−0.13
	globulin	1.45	1.43	+0.02
	total	4.35	4.46	−0.11
Nephrosis	albumin	2.66	2.60	+0.06
	globulin	2.17	2.24	−0.07
	total	4.83	4.84	−0.01
Plasma saline	albumin	2.82	2.87	−0.05
	globulin	2.06	2.18	−0.12
	total	4.88	5.02	−0.17
Plasma saline	albumin	2.49	2.65	−0.16
	globulin	2.13	1.96	+0.17
	total	4.62	4.61	+0.01
Cardiac with edema	albumin	2.77	2.56	+0.21
	globulin	2.17	2.15	+0.02
	total	4.94	4.71	+0.23
Plasma saline	albumin	1.79	1.77	+0.02
	globulin	1.53	1.31	+0.22
	total	3.32	3.08	+0.24
Nephrosis	albumin	2.37	2.29	+0.08
	globulin	1.83	1.73	+0.10
	total	4.20	4.02	+0.18
Mixed plasma	fibrinogen	0.41	0.45	−0.04
Mixed plasma	fiibrinogen	0.67	0.65	+0.02
			Average Deviation	+0.02

on a few dilutions of these and the average equivalent determined. The average equivalent for albumin was 12.9 and for globulin 13.1.

Table I shows the comparison between the colorimetric method based on the tyrosine equivalents just given, and the protein based on nitrogen determinations.

SUMMARY

A modification and simplification of the Wu colorimetric method for the determination of plasma proteins is described. It has been found that not only a maximum chromogenic value but approximately equal tyrosine equivalents for the plasma proteins are obtained by heating these for a half hour with 10 per cent sodium hydroxide.

On the basis of these similar tyrosine equivalents, modifications are presented for the determination of total protein of plasma and spinal fluid which give values of sufficient clinical accuracy, irrespective of the protein partition.

A table gives a comparison of protein values obtained by this method and values based on nitrogen content.

ADDENDUM

Occasionally we have had plasma specimens from which the globulin failed to centrifuge out satisfactorily. In practice, we now determine the total protein on 0.1 c.c. of plasma heated a half hour with 0.5 c.c. of 10 per cent sodium hydroxide solution. The fibrinogen is separated as outlined and the supernatant fluid is drawn off completely; 2 c.c. of water and 4 c.c. of saturated ammonium sulphate are added and the mixture warmed at 37° C. for fifteen minutes. The globulin precipitate is removed by filtration through a pleated filter, the filtrate being returned onto the paper until clear. Albumin is determined on 2 c.c. of the filtrate. The globulin is figured by subtracting the sum of the fibrinogen and albumin from the total protein.

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STUDIES IN THE SEROLOGY OF SYPHILIS*

XII. Modifications in the Technic and Interpretation of the Wassermann Reaction

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DESPITE the general adoption of various precipitation tests, the Wassermann reaction remains the one test most widely used in the laboratory diagnosis of syphilis. Nevertheless, there is widespread disagreement as to the optimum technical procedure. Changes which have been suggested by various serologists from time to time have not been universally adopted, with the result that the technic employed in different laboratories may vary considerably, and with it, of course, the sensitivity and specificity of the results obtained. Moreover, certain points of view as to the interpretation of results have become so firmly entrenched by constant reiteration in the enormous literature as to appear almost axiomatic to the present-day clinician and serologist, despite considerable evidence that some of these may be erroneous.

The present paper represents an attempt to determine, on the basis of the results obtained in this laboratory, the optimum adjustment of some of the factors on which serologists disagree; the temperature and duration of incubation, the number of antigens to be used, the proper degree of antigen sensitization, and the methods to be used in the standardization of the reagents. There are included some observations on the interpretation of Wassermann results, specifically, (1) the questionable value of 1+, 2+, 3+, and 4+ reports as a quantitative scale of positivity, (2) the incidence of false positive Wassermann reactions in diseases other than syphilis, and (3) the nature of the active substance in syphilitic serum, so-called reagin.

A. UNNECESSARY MULTIPLICATION OF ANTIGENS

Many laboratories test each serum with two, three, or even four different antigens. Two advantages might conceivably attach to such a procedure; but as will be shown, neither is realized in practice. Such reports as

Wassermann Reaction
Plain antigen = negative
Cholesterolized antigen = positive
Acetone—insoluble antigen = doubtful

serve only to confuse the clinician, and destroy confidence in the particular laboratory.

In the first place, the use of multiple antigens does not serve as a check against false positive reactions. Given a serum which is positive with one antigen, and negative with another, this usually means only that the two antigens differ in sensi-

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tivity; that is, the serum does contain syphilitic reagin, but the amount of reagin in the particular serum is less than the threshold value for the weaker antigen. The incidence of biologic false positive Wassermann reactions, even with the most sensitive antigen available is so small that a persistently positive reaction may be taken as prima facie evidence of the presence of syphilitic infection (see Section C). It might be said that the use of multiple antigens is a check against such laboratory errors as failure to add complement, the use of an anticomplementary antigen, etc. No well-run laboratory uses antigen in more than a small fraction (¼-⅛) of its anticomplementary dilution; while the best method of controlling the other types of error is to use several dilutions of serum. This procedure serves not merely as a mutual check, but as a semiquantitative titration of the reagin content of the serum.

On the other hand, one might argue that the use of several antigens makes doubly certain the detection of a syphilitic serum. It is true that if one tests a large number of syphilitic sera with several antigens, there will be a considerable difference in the number detected by the individual antigens (Table I). Nevertheless, as is seen in Table I, such differences are not haphazard, but depend solely upon the absolute sensitivity of the antigen. We find no evidence that antigens differ in their selective affinity for particular clinical conditions.

To demonstrate these points, the sera of 258 syphilitic patients undergoing active treatment for syphilis were examined with five different antigens. A second group of 265 similar cases were tested by the antigen used in this laboratory (Protocol 1 (e)) and by the Kolmer antigen supplied by the Digestive Ferments Co. The preparation of the antigens, and the technic of the test, are described in Protocol 1. The results are listed in Table I. As is there shown, the most sensitive antigen detects *every* serum picked out by the weaker antigens, *plus* a certain number which they missed because of their lower sensitivity. In not a single case did a "weak" antigen pick up a serum missed by the usually more sensitive antigen. It is obvious that no virtue attaches to the use of 2, 3, or 4 antigens. One need only select the most sensitive of the lot, and use it alone.

PROTOCOL I

Preparation of Antigens.—a. Fifty grams of dried powdered beef heart (Difco) were extracted with 250 c.c. of ether for fifteen minutes at 37° C. The ether filtrate was discarded and the moist powder was washed on the filter with 100 c.c. of warm ether, dried, and extracted at 37° C. for four days with 250 c.c. of 95 per cent ethyl alcohol. After filtration, the moist powder was washed with 100 c.c. of alcohol, and the combined alcoholic filtrate and washings evaporated down to 75 c.c. Seventy-five c.c. of absolute alcohol were then added.

b. The extract obtained as in (a) was sensitized with 0.2 per cent cholesterol.

c. Fifty grams of dried powdered beef heart (Difco) were extracted with 250 c.c. of alcohol for four days at 37° C. The alcoholic filtrate was evaporated down to dryness, and the soft residue washed 3 times with 100 c.c. of hot acetone. The acetone extracts were discarded; and the hard waxy residue dissolved in 5 to 10 c.c. of ether. Then 200 c.c. of hot 95 per cent alcohol were added, the mixture evaporated on the steam bath down to 150 c.c., and filtered. Cholesterol was added to a concentration of 0.4 per cent.

d. The extract obtained as in (a) was sensitized with 0.6 per cent.

e. The extract obtained as in (a) was sensitized with 0.8 per cent cholesterol and 0.6 per cent sitosterol, dissolved by boiling. This antigen (e) is the one in routine use at the Johns Hopkins Hospital. It has since been found that the antigen containing 0.6 per cent cholesterol and 0.6 per cent corn germ sterol yields identical results, with the added advantage that it can be

used in a flocculation test as well (Paper VIII of this series, J. Lab. & Clin. Med. **17**: 787, 1932). Both antigens can be obtained from the Digestive Ferments Co., Detroit.

f. Kolmer antigen supplied by the Digestive Ferments Co.

Technic of Wassermann Test Used in All the Experiments Included in This Paper.—The clear serum was inactivated at 56° C. for twenty minutes, and 0.1, 0.1, and 0.05 c.c. respectively were added to three tubes. Complement was prepared by pooling the blood of at least 5 pigs, allowing it to clot overnight at ice box temperature, and centrifuging. Two-tenths c.c. of a 1:12 dilution in 0.85 per cent NaCl were added to all three tubes.

Antigen was used in a 1:200 dilution, prepared by dropping one volume of antigen slowly with shaking, into 200 volumes of salt solution.* In the case of antigen e, the excess of sensitizer is redissolved by heating the antigen at 56° for a few minutes before diluting. Two-tenths cubic centimeter of the dilution are added to Tubes 2 and 3, Tube 1 serving as an anticomplementary control.

Two-tenths cubic centimeter of salt solution are added to Tube 1, making a total volume of 0.5, 0.5, and 0.45 c.c. respectively in the three tubes.

The tests were then placed in the ice box for four hours, followed by one-half hour incubation at 37° C. Four-tenths cubic centimeter of a sensitized cell suspension were then added and the results read in fifteen to twenty minutes.

The sensitized cells were prepared by washing citrated sheep's blood with 10 volumes of salt, and diluting the measured cells with 32 volumes of salt solution. The minimal dilution of amboceptor which will cause the hemolysis of this suspension in one-half hour as then determined (0.2 c.c. of amboceptor + 0.2 c.c. 3 per cent cells + 0.2 c.c. 1:12 complement + 0.4 c.c. salt solution). The 3 per cent cell suspension was then sensitized with an equal volume of amboceptor dilution containing 2½ to 3 times this minimal hemolytic quantity, and 0.4 c.c. of the resultant 1½ per cent cell suspension used in the test.

B. THE SUPPOSED DANGER OF FALSE POSITIVE REACTIONS WITH HIGHLY SENSITIZED ANTIGENS

The use of cholesterol to increase the sensitivity of Wassermann antigens is a generally employed procedure. Nevertheless, serologists have been unaccountably cautious in taking full advantage of its sensitizing properties, the majority of laboratories using it in a concentration of only 0.2 per cent. Yet, as may be seen from Table I and Fig. 1, supersaturation with cholesterol, up to a concentration of 0.8 per cent, does not exhaust the possibilities of sensitization. A plain alcoholic antigen yielded 34 per cent of positive Wassermann reactions among a known syphilitic population who were under active treatment for syphilis; the same antigen containing 0.2 per cent cholesterol yielded 40 per cent, with 0.6 per cent cholesterol, 57 per cent, and with 0.8 per cent cholesterol plus 0.6 per cent sitosterol in addition, 70 per cent of positive tests among the same patients. In a general hospital population, excluding those under specific treatment for syphilis, the last named antigen detected 60 to 65 per cent more syphilitic sera than the same antigen without sensitizer (Table II).

The failure of serologists to take advantage of maximum sensitization as an aid to diagnosis is due to a widespread belief that highly cholesterolized antigens tend to yield false positive reactions. This impression I do not believe to be justified. It is true that a highly sensitized antigen is more anticomplementary than the plain alcoholic extract. Nevertheless, if due allowance is made for this increased anticomplementary activity by using the antigen in a small fraction of its anticomplementary concentration (less than ⅛), the following results indicate

*See footnote on page 829. A more sensitive antigen dilution can be obtained by reversing the order of dilution, dropping the salt solution *slowly* with shaking into the antigen.

that an antigen containing even 1.4 per cent of sensitizing sterols (Protocol 1 (e)) does not yield a significant proportion of false positive reactions; and that as already stated, a persistently positive Wassermann reaction with such an antigen is to be taken as prima facie evidence of syphilitic infection.

TABLE I A

SHOWING THAT THE MORE SENSITIVE ANTIGEN WILL ALMOST INVARIABLY DETECT THOSE SERA PICKED UP BY WEAKER ANTIGENS: I. E., ANTIGENS DO NOT HAVE HAPHAZARD SELECTIVITY FOR PARTICULAR SERA OR FOR PARTICULAR CLINICAL CONDITIONS

	WASSERMANN RESULT WITH											
NO. OF SERA	PLAIN ALCOHOLIC EXTRACT		A + 0.2 % CHOLESTEROL		ACETONE INSOLUBLE + 0.4 % CHOLESTEROL		A + 0.6 % CHOLESTEROL		A + 0.8 % CHOLESTEROL + 0.6 % SITOSTEROL		EAGLE FLOCCULATION TEST[1]	
61	0		0		0		0		0		0	
17	0		0		0		0		0		+	
11	0		0		0		0		±		+	
2	0		0		0		0		±		0	
3	0		0		0		0		+		0	
1	0		0		0		0		+		+	
17	0		0		0		0		+		+	
18	0		0		0		±		+		+	
13	0		0		0		+		+		+	
2	0		0		0		+		+		±	
10	0		0		±		+		+		+	
6	0		±		±		+		+		+	
4	0		±		+		+		+		+	
5	0		+		+		+		+		+	
5	±		±		+		+		+		+	
1	±		+		+		+		+		0	
14	±		+		+		+		+		+	
68	+		+		+		+		+		+	
Total	+	±	+	±	+	±	+	±	+	±	+	±
258	68	20	88	15	97	16	128	18	167	13	188	3
	26%	8%	34%	6%	37%	6%	50%	7%	65%	5%	73%	1%

TABLE I B

WASSERMANN RESULT WITH

NO. OF SERA	KOLMER ANTIGEN		ANTIGEN OF PROTOCOL 1 (E)	
88	0		0	
28	0		±	
41	0		+	
53	±		+	
55	+		+	
265	+	±	+	±
	55	53	147	28
	28%	20%	55%	10%
	48%		66%	

1. Of 189 applicants for the blood transfusion donor list tested by such an antigen using the technic outlined in Protocol 1, only one gave the slightest trace of fixation: that one case was found to have a darkfield positive chancre.

2. Nine hundred and forty-six white, comparatively well-to-do patients in the private wards and in a semi-private outpatient department, in whom the incidence of syphilis is lower than in any other hospital group, were also tested by this

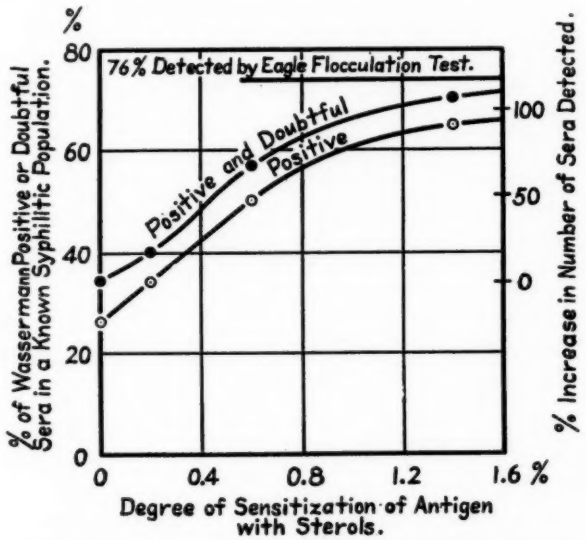

Fig. 1.—Showing the effect of increasing sensitization upon the sensitivity of a Wassermann antigen.

strongly sensitized antigen. The results are outlined in Table III. There were 19 positive and 7 doubtful Wassermann reactions in the entire group.

The doubtful reactions are omitted from consideration, as they include all tests with incomplete hemolysis, whether that was due to technical error or actual

TABLE II

SHOWING THAT A STRONGLY SENSITIZED ANTIGEN DETECTS 60 PER CENT MORE WASSERMANN POSITIVE SERA IN A GENERAL HOSPITAL POPULATION THAN A PLAIN UNSENSITIZED EXTRACT.

	NUMBER OF CASES	WASSERMANN RESULT WITH PLAIN ANTIGEN			WASSERMANN RESULT WITH EXTRACT SUPERSATURATED WITH STEROLS		
	1039			0			0
	18			0			±
	30			0			+
	2			±			±
	15			±			+
	76			+			+
Total	1180	+	±	0	+	±	0
		76	17	1087	121	32	1039
		65%	15%	92%	10.3%	2.7%	87%
		8%			12.9%		

fixation. Such reports are always accompanied with the request that the test be repeated. Of the positive tests, two were obviously technical errors, due to some one of many possible factors (inadvertent omission of complement; mislabelled specimens, dirty tube, etc.); for a simultaneous precipitation test and all subsequent Wassermann reactions were entirely negative. Such technical false positive (or doubtful) reactions can not be properly considered due to the nonspecificity of the antigen, for they are encountered with varying frequency in any large laboratory, no matter what the technic or antigen used, and are clearly not due to the antigen as such. Of the remaining 17 positive reactions, 8 were in cases with definite clinical manifestations of syphilis. In 4, there was strong presumptive evidence of syphilitic infection. In one case the test was not repeated. There remained only 4 cases in this entire series of 946 in which there was a persistently positive Wassermann reaction in the absence of any clinical findings or history suggestive of syphilis. In view of the fact that the serologic finding is the only evidence of infection in fully one-third of all Wassermann posi-

TABLE III

SHOWING THAT AN ANTIGEN CONTAINING AS HIGH AS 1.4 PER CENT SENSITIZING STEROLS DOES NOT YIELD PERSISTENT POSITIVE REACTIONS IN NONSYPHILITIC INDIVIDUALS: BASED ON 946 PRIVATE PATIENTS

WASSERMANN NEGATIVE	WASSERMANN POSITIVE—19 CASES				
	TECHNICAL ERROR	CLINICAL EVIDENCE OF SYPHILIS IN 8 CASES	SYPHILIS PROBABLE IN 4	NOT REPEATED	PERSISTENTLY POSITIVE IN ABSENCE OF HISTORY OR CLINICAL SIGNS
920	2	6 neurosyphilis 1 aortic insufficiency 1 optic atrophy	2 History of penile lesion and treatment 1 Probable aortitis 1 Husband had tabes	1	4

tive cases in our dispensary,[2] it is quite probable that these were similar cases of latent syphilis. Indeed, of 50 positive Wassermann reactions obtained with a plain nonsensitized extract on patients in the general dispensary, almost exactly the same proportion (30 per cent) were in patients who gave no history or clinical evidence of syphilis. Moreover, the 4 cases in question all gave a persistent and strongly positive precipitation test. It would therefore appear that sensitization of an antigen, per se, does not cause nonsyphilitic patients to give a positive Wassermann reaction in more than a very small and indeterminate fraction of one per cent of the cases.

The cases in the general hospital and dispensary population which yielded a negative or doubtful Wassermann reaction when tested by a plain extract, and a persistently positive Wassermann when tested by a strongly sensitized antigen (3.8 per cent of the total group), thus can not be interpreted as nonspecific false positive reactions due to excessive sensitization, but must be considered as syphilitic. The surprising number of such disagreements (3.8 per cent of a general hospital and dispensary population and 30 to 35 per cent of the total positives encountered) simply reflects the fact that the reagin titer of syphilities varies between

wide limits (Fig. 2). If one titrates a group of syphilitic sera one finds that a few contain 100, 200, or even 400 units of reagin. Such sera will be Wassermann positive with almost any antigen. A larger number, containing intermediate quantities (20, 30, or 50 units) will be Wassermann positive with moderately sensitive antigens, but may yield negative or doubtful results with plain extracts. There remain a large proportion of known syphilitic sera, with comparatively small quantities of reagin, 0 to 10 units, which are usually missed by any save a highly sensitive antigen, and even then, may yield inconsistent results upon repeated tests.

As is shown in Fig. 2, the same variation in reagin titer is encountered in a group of Wassermann positive patients who had not received previous treatment for syphilis. A considerable proportion of these (20 to 40 per cent) would be missed by weakly sensitized antigens. We have already shown that maximal sen-

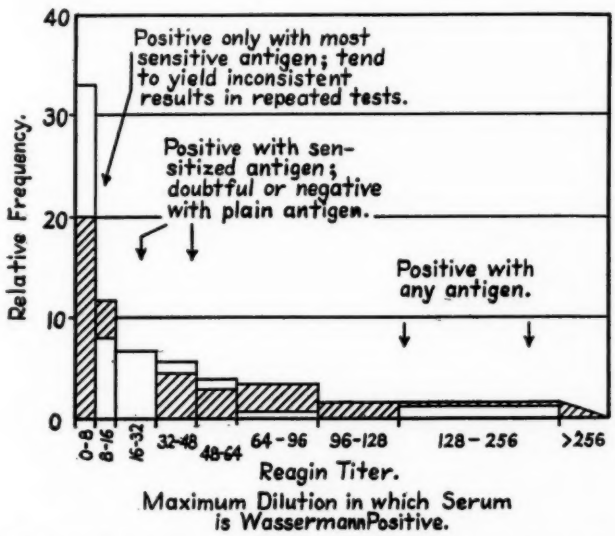

Fig. 2.—Variations in reagin titer among Wassermann positive sera. White area indicates known syphilitics under active treatment (146 cases). Oblique lines indicate previously untreated (120 cases).

sitization, even up to 1.4 per cent, while bringing about an increased sensitivity, does not predispose to false positive reactions to any significant extent; and there is thus no reason for the continued use of plain alcoholic extracts as Wassermann antigens.

C. THE INADEQUACY OF THE WATER-BATH WASSERMANN REACTION

It is now twenty-two years since Jacobsthal[3] suggested that the sensitivity of the Wassermann reaction could be increased by incubating the mixture of complement, antigen, and serum at ice box temperature prior to the addition of cells. As I have shown elsewhere,[4] this increased sensitivity depends upon at least four factors: (1) the prolonged incubation period, making for greater fixation of complement; (2) a spontaneous deterioration of complement; (3) a less marked inhibition of fixation by serum protein at the lower temperature; (4) a greater nonspecific destruction of complement by antigen.

The difference in sensitivity between a thirty minute water-bath test, and a

TABLE IV

THE COMPARATIVE SENSITIVITY OF THE ONE-HALF HOUR WATER-BATH AND FOUR-HOUR ICE BOX TEST IN A KNOWN SYPHILITIC POPULATION UNDER ACTIVE ANTISYPHILITIC TREATMENT

PLAIN ALCOHOLIC EXTRACT							SENSITIZED ANTIGEN						
NO. OF CASES	WASSERMANN RESULT BY WATER-BATH INCUBATION ½ HOUR			4-HOUR ICE BOX + ½-HOUR WATER-BATH			NO. OF CASES	WASSERMANN RESULT BY WATER-BATH INCUBATION ½ HOUR			4-HOUR ICE BOX + ½-HOUR WATER-BATH		
170	0			0			78	0			0		
17	0			±			13	0			±		
3	±			±			0	±			±		
26	0			+			58	0			+		
11	±			+			21	±			+		
31	+			+			88	+			+		
Total	+	±	0	+	±	0		+	±	0	+	±	0
	31	14	213	68	20	170		88	21	149	167	13	78
	12%	6%		26%	8%			34%	8%		65%	5%	
	18%			34%				42%			70%		

test with the same reagents, but in which the tube is placed for four hours in the ice box before the water-bath incubation, is truly surprising. Of 258 known syphilities only 109 were detected by a water-bath test with the most sensitive antigen available; while 180 were detected by the combined four hour ice box and one-half hour water-bath test.

In not a single instance did the water-bath test detect a serum missed by the ice box incubation. Despite the greater sensitivity of the ice box test, it has not been universally adopted. As recently as 1928, of the seven serologists who carried out Wassermann reactions in the competitive League of Nations Conference, only one did a preliminary ice box incubation, and then only for one hour. At the 1930 Conference, of 7 Wassermann technics, 6 were water-bath tests, and one involved a brief incubation at room temperature. Moreover, many laboratories which have adopted some type of cold incubation procedure continue to carry out a duplicate water-bath test, despite the fact that nothing is gained thereby.

As in the case of sensitization, this cautiousness can be attributed to a widespread, but in my opinion, mistaken impression that the ice box test yields false positive reactions. An eighteen hour ice box test proved occasionally unsatisfactory in this laboratory not because of biologic false positive reactions, but because of spontaneous deterioration of complement under the conditions of the test. In my own experience, however, the four-hour ice box test followed by one-half hour at 37° C. is entirely reliable, and as is shown by the data of pages 824 to 826, does not yield biologic false positive reactions; at least, if it does, the incidence is certainly less than an insignificant fraction of one per cent.

D. THE OPTIMUM DURATION OF INCUBATION

Granted that a preliminary ice box incubation makes for a very significant increase in the sensitivity of the Wassermann reaction, what should be its duration?

A number of serologists have recommended that the antigen-serum-complement mixture be incubated eighteen hours in the ice box in order to obtain the maximum degree of complement fixation.[5] They consider that the increased sensitivity thus attained more than compensates for the inconvenience of a two-day reaction. In this laboratory, however, such an overnight test has proved both unnecessary and undesirable, for the reasons discussed below.

1. We have not been able to confirm the finding that overnight incubation is necessary for maximum sensitivity. It is true that if a group of sera from patients under active antisyphilitic treatment is tested with a plain alcoholic beef heart extract, by the technic described in Protocol 1, incubating duplicate series of tests for varying periods of time in the ice box, there is a progressive and striking increase in the degree of complement fixation and in the number of positive reactions as the incubation period is prolonged. Thus, among 135 patients tested by one-half hour incubation at 37° C., there was a total of 16 per cent positive and doubtful reactions, increased to 37.7 per cent by preliminary two hours' incubation at 0° to 6° C., to 47 per cent by preliminary four hours at 0° to 6° C., and to 62.2 per cent by overnight ice box incubation (Fig. 3). Fully 24 per cent of the ultimately positive reactions would have been missed by a preliminary four hour ice box incubation. If, however, a duplicate series of tests is carried out at the same time, under the same conditions, using the same basic antigen supersensitized with sterols (Protocol 1 e*), quite different results are obtained. Instead of a progressive increase in the number of positive reactions over the entire range of one-half to eighteen hours' incubation, there is a sharp increase in sensitivity within the first two to four hours; but *further incubation has very little effect on the number of sera detected.* The fixation of complement is so accelerated by the highly sensitized antigen that the great majority (97.4 per cent) of those sera which are positive or doubtful after eighteen hours in the ice box are already so after four hours in the ice box + one-half hour at 37° C. (Fig. 3).

It follows that *with a sufficiently sensitized and properly diluted antigen,* the difference in the sensitivity of the overnight as compared with the four hour ice box test is not sufficient to justify the inconvenience of the longer incubation, particularly in view of the undesirable features of the latter described in the following paragraphs.

2. Whatever the cause for the anticomplementary action of serum, whether bacterial contamination, chemical impurities introduced into the blood, or an intrinsic property of certain sera, destruction of complement is a process in time: the longer the incubation period, the more complement is destroyed. It follows that sera apparently free from such properties when tested by incubation at 37° C. for one-half hour, may appear definitely so if the water-bath incubation is preceded by four hours' incubation at 0° to 6° C.; more to the point, *the number of anticomplementary reactions in an overnight ice box test is necessarily higher than that observed after four hours,* all other factors remaining the same. By the technic used in this laboratory, employing 2½ units of complement, 1 per cent of the general

*The antigen in this experiment was prepared by dropping two volumes of salt solution, slowly and with shaking, into 1 volume of antigen. After a two- to five-minute interval, the remaining 198 volumes of salt solution were added. Such a dilution is more turbid and more sensitive than that prepared by dropping the antigen into the salt solution. Otherwise, the technic was that described in Protocol 1.

hospital dispensary population and 2.5 per cent of those receiving antisyphilitic therapy yield anticomplementary results when tested by a four hour ice box, one-half hour 37° test: with overnight ice box incubation, these are increased to 3.5 and 6.5 per cent respectively. Any attempt to overcome this tendency, as by increasing the amount of complement, tends to defeat the very purpose of the overnight test, by making the reaction less sensitive.

3. Although the hemolytic activity of fresh complement from healthy guinea pigs remains practically unchanged after overnight incubation as a 1:30-1:60

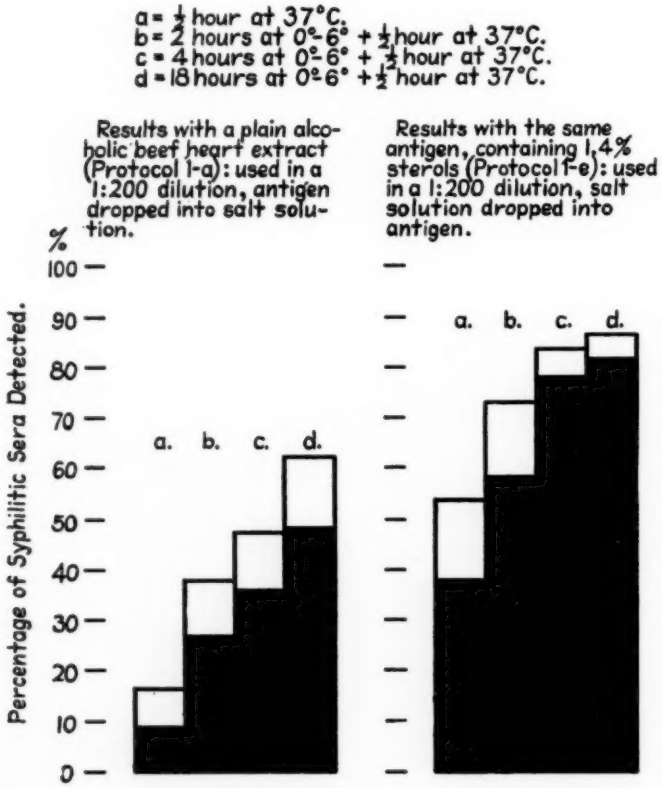
a = ½ hour at 37°C.
b = 2 hours at 0°-6° + ½ hour at 37°C.
c = 4 hours at 0°-6° + ½ hour at 37°C.
d = 18 hours at 0°-6° + ½ hour at 37°C.
Results with a plain alcoholic beef heart extract (Protocol 1-a): used in a 1:200 dilution, antigen dropped into salt solution.
Results with the same antigen, containing 1.4% sterols (Protocol 1-e): used in a 1:200 dilution, salt solution dropped into antigen.
Percentage of Syphilitic Sera Detected.
%
100
90
80
70
60
50
40
30
20
10
0
a. b. c. d.
a. b. c. d.

Fig. 3.—Showing that *with a highly sensitized antigen*, the overnight ice box test is not appreciably more sensitive than the four hour ice box plus one-half hour water-bath test. Solid black indicates positive reaction (no hemolysis). White area indicates doubtful reaction (partial hemolysis).

dilution, particularly in the presence of serum, complement from sick, anemic, or poorly nourished pigs may, in our experience, occasionally deteriorate so markedly during the overnight incubation as to invalidate the results of an entire series.

4. A strongly sensitized antigen is so anticomplementary upon overnight incubation that a quantitative titration of the serum is usually impossible. The qualitative test with whole serum, or serum diluted with two, three, or four volumes of salt solution may be quite satisfactory, inasmuch as the serum masks the anticomplementary action of the antigen. If, however, the test is performed upon serial dilutions of serum in order to determine its reagin content quantitatively, the antigen is usually so anticomplementary as to preclude the reading of the test

in the higher serum dilutions. The use of weaker, that is, less strongly sensitized antigens, destroys the very purpose of the overnight incubation.

For these reasons, we have adopted as the routine in this laboratory a four hour ice box incubation, followed by one-half hour at 37° C. *before* the addition of sensitized cells, using an antigen containing 1.4 per cent sterols and diluted with 200 volumes of salt solution in such a manner as to ensure maximum sensitivity (see footnote p. 829).

E. THE "TITRATION" AND "STANDARDIZATION" OF WASSERMANN REAGENTS

Every reagent used in the Wassermann reaction affects the sensitivity and specificity of the results obtained; as a result, the most important duty of the Wassermann technician is to make the necessary adjustments in the reagents. The antigen lipoid must be sufficiently concentrated and sensitized to ensure the detection of weakly positive sera; yet it must not be hemolytic or anticomplementary in the dilution used. The cell suspension must be sufficiently heavy to ensure a ready distinction between a positive (no hemolysis) and a negative result (hemolysis); the varying fragility of the cells must be properly compensated for. The complement and amboceptor activity must be controlled, as too much complement (or amboceptor) obscures weakly positive sera; while if there is too little complement or amboceptor, or if for some reason the complement deteriorates under the conditions of the test, the cells will fail to hemolyze in positive and negative sera alike. Moreover, the level of sensitivity must be kept fairly constant, else the results with sera of identical reagin content will vary from day to day.

To safeguard against any of these eventualities, there has been developed a maze of titrations and cross-titrations which constitute the technician's stock-in-trade. Without wishing in the slightest degree to detract from the necessity of rigid control, I do believe that these are unnecessarily complex; and that the technician can save himself considerable labor of reduplication by keeping in mind a few simple fundamentals.

1. *Antigen Titrations.*—When antigens were prepared by simple alcoholic extraction of wet tissue, it was unavoidable that the alcohol concentration and lipoid content of final antigen were a matter of chance, and that the extract should be both anticomplementary and hemolytic. Of necessity such antigens had to be carefully titrated so as to be used in a dilution which, although possessing neither of these undesirable properties, should yet be sufficiently "antigenic" to detect positive sera. By modern methods of preparation, however, such repeated titrations become unnecessary. Antigens can be prepared, not occasionally, but uniformly, which are not demonstrably hemolytic, are insignificantly anticomplementary, and yet are so active that their antigenic activity, after an initial test, need not be checked again. They can be used every day in the same dilution, with the assurance that one is using from 10 to 50 antigenic "units," and yet that one is 40 times removed from the danger zone of an anticomplementary antigen. A recommended method of preparing such an antigen is that described in Protocol 1 (e).

2. *Complement and Amboceptor Titrations.*—Individual guinea pigs vary enormously in the complement (hemolytic) activity of their sera. The technician attempts to correct for this variation by determining the minimal hemolytic

quantity of complement, and by using some arbitrary but fixed number of such units in the actual reaction. Such a calibration ignores completely the fact that sheep cells vary by as much as 300 to 500 per cent in their susceptibility to hemolysis. Thus, comparing the bloods of the four sheep used in this laboratory, the most resistant requires 3½ times as much complement per unit cell volume as the least resistant, all other factors remaining constant. Obviously a calibration of complement in which cells are used as the yardstick, is, if not futile, quite inadequate. The hemolytic unit of complement may be 0.2 c.c. of a 1:30 dilution one day, and apparently the same two weeks later. Yet, because two different cell suspensions are used as the test object, the one unit may contain 2 to 3 times as much active complement as the other, with corresponding undesirable effects upon the sensitivity of the test.

There is another even more obvious error in the usual method employed to determine the complement "unit." Using a given cell suspension and a given quantity of complement, say 0.2 c.c. of a 1 to 10 dilution, the hemolytic quantity of amboceptor (unit) is first determined. An arbitrary multiple of amboceptor units, say three, are then added to the cells. With this sensitized cell suspension, the minimal hemolytic quantity of complement is then determined. This second titration of complement is meaningless, as it is predetermined by the number of amboceptor units used in the sensitization of the cells. If one unit of amboceptor had been used, the 0.2 c.c. of a 1:10 dilution of complement are barely hemolytic, and are thus one unit; with two units of amboceptor, the complement is found to be hemolytic in 1:20 dilution, and 1:10 complement will be two units; if 3 units of amboceptor are used, the complement may hemolyze the cells up to a 1:25 dilution, and 1:10 complement will be two and a half units. Clearly, this complement titration, upon which so much stress is laid as a means of standardizing the reaction, is in reality determined solely by the amount of amboceptor used to sensitize the cells.

The author believes the following method of calibration to be at least as reliable as any now in use, with the added advantage that it eliminates to a large extent the necessity for such complex cross-titrations as just outlined.

a. Use pooled complement of at least 3 or 4 pigs, and preferably more. Use it always after allowing it to clot for twenty-four hours in the ice box. In this manner, the variations between the activity of individual pigs can be minimized, and the complement taken as a more or less standard reagent.

b. Use a constant quantity of pooled complement every day; e. g., 0.2 c.c. of a 1:10 dilution (+0.2 c.c. of antigen dilution, +0.2 c.c. of the serum dilution).

c. Correct for the varying fragility of the cell suspensions by determining the minimal hemolytic unit of amboceptor each day, using the same amount of complement and cells in the titration as is to be used in the test. Sensitize the cell suspension with some arbitrary multiple of this unit, e. g., 3. With such a cell suspension, one will always be using 2 or 3 units of complement in the test: that is, the cell suspension is hemolyzed by 0.2 c.c. of 1:25 complement, as compared with the 1:10 complement used in the test. This provides an ample margin of safety for slightly anticomplementary sera, slight deterioration of complement, etc.

d. Test for deterioration of complement under the conditions of the test by

determining the minimal hemolytic dilution before and after the proper period of incubation. Thus, for the technic outlined in Protocol 1: to 0.2 c.c. of 1:10, 1:20, 1:25, 1:30 and 1:40 complement add 0.4 c.c. of saline and incubate for four hours in the ice box and one-half hour at 37° C. Add 0.4 c.c. of the cell suspension prepared as described in the preceding paragraph, and read hemolysis after one hour. If the readings differ appreciably from those obtained with freshly diluted complement there has been deterioration of complement. Slight deterioration in the 1:25 dilution or higher will have no effect in the test. Thus,

Complement dilution	1:10	1:20	1:25	1:30	1:40
Reading of hemolysis with freshly diluted complement	4	4	4	2	1
With complement diluted and incubated	4	4	2	2	0

implies minimal deterioration in the 1:10 dilution, and a satisfactory complement. On the other hand, a reading such as

	1:10	1:20	1:25	1:30	1:40
Freshly diluted complement	4	4	4	2	0
Complement after incubation	4	2	0	0	0

implies considerable deterioration of complement, and means (1) that the results in the particular series of tests must be read with the greatest caution, and (2) that the guinea pigs from which the complement was obtained must be given a rest or replaced.

F. THE SO-CALLED 1+, 2+, 3+, AND 4+ (COMPLETELY POSITIVE) WASSERMANN REACTIONS

It has become traditional to report Wassermann results on a scale of plus marks. On this basis, 0 is a negative reaction, in which the complement remains free, and the cells hemolyze completely; 1+ is a "weak positive" reaction, in which the complement has been fixed to a slight extent, so that the tube remains hazy with undissolved cells; 2+ and 3+ represent increasing degrees of positivity, with more and more complement fixed, and decreasing degrees of hemolysis; 4+ is, on this scale, the strongest type of reaction, in which there is no detectable hemolysis, due to "complete" fixation of the complement.

TABLE V

SERUM DILUTION

	1	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Results with 8 "completely positive" (4+) sera	4+	4+	4+	4+	4+	4+	4+	4+
	4+	4+	2+	0	0	0	0	0
	4+	4+	4+	4+	4+	3+	0	0
	4+	4+	4+	4+	4+	0	0	0
	4+	4+	4+	4+	0	0	0	0
	4+	4+	4+	4+	4+	4+	4+	0
	4+	4+	0	0	0	0	0	0
	4+	4+	4+	4+	4+	1+	0	0

Actually, as Vedder[6] and Keidel[7] have shown, such a quantitation is entirely illusory, and does not even begin to reflect the total possible variation in the reagin content of syphilitic sera. If one selects a group of "4+" sera at random, and determines the maximum dilution in which this positive reaction can be obtained, one finds that there is a truly enormous variation, illustrated in Table V and Fig. 4.

Some of the sera contain barely enough reagin to be positive with whole serum; some can be diluted 5 fold; while some continue to give a 4+ reaction even when diluted with 10, 40, or several hundred volumes of saline.

The degree of positivity of syphilitic sera can thus be expressed on a titer scale analogous to that of the Widal reaction (Fig. 4). The term 4+ does not mean a

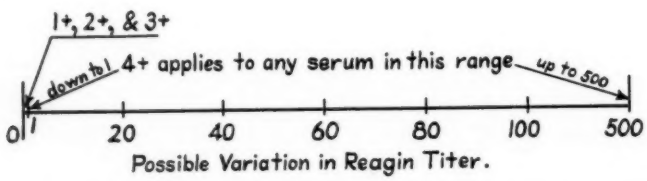

Fig. 4.—Showing the inadequacy of 1+, 2+, 3+, and 4+ reports as a quantitative scale of positivity.

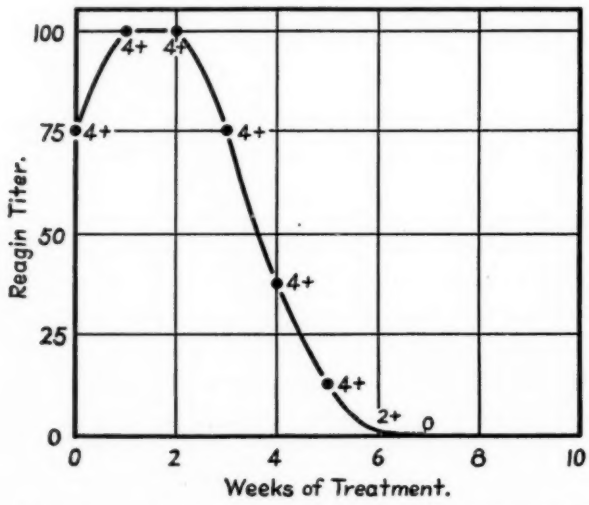

Fig. 5.—Serologic response of a patient with early syphilis to treatment with arsphenamine.

strongly positive reaction, but applies to any serum which is Wassermann positive, whether that serum contains one or 400 units of reagin; while the terms 1+, 2+, 3+ upon which so much stress has been laid as a quantitative measure of positivity, actually cover a very minute and insignificant portion of the entire possible variation.

A frequent clinical observation becomes intelligible in the light of this quantitative aspect of the Wassermann reaction. A patient with early syphilis placed under specific therapy may continue to give a 4+ Wassermann for four, eight, twelve, or even sixteen weeks; and then quite suddenly, this "strong" Wassermann reaction breaks and becomes negative, frequently without passing through

the intermediate stages of 1, 2, or 3+. As a matter of fact, such a series of weekly Wassermann reactions (e. g., 4, 4, 4, 4, 4, 4, 4, 4, 4, 2, 0) gives not the slightest clue to the actual course of events. The patient's serum may have had an original titer of 200, i. e., 200 times as much reagin as is necessary to give a 4+ reaction; under specific therapy, this may have fallen, week by week, to 100, 50, 25, 10, 5 with the routine reaction remaining 4+ all the while. It is only in the last part of the curve, when the serum reagin has almost completely disappeared, that the Wassermann breaks, and one obtains the long awaited serologic evidence of improvement in the appearance of 3, 2, 1+, or negative results (Fig. 5).

It follows that

1. Reports should be made, not as 0, 1+, 2+, 3+, 4+, but, as the League of Nations Serological Conference suggested in 1928, as negative (complete hemolysis), positive (no hemolysis), or doubtful (partial hemolysis).

2. A doubtful report in a patient not known to have syphilis may mean either that the serum contains minute quantities of reagin, but not enough to prevent hemolysis entirely, *or,* that the reagents were not properly adjusted. It calls for careful serologic and clinical check-up.

3. If a truly quantitative test of a positive serum is desired, it is necessary to determine the maximum dilution in which it continues to yield a positive result. The rate with which this Wassermann titer falls toward negative under treatment may be of distinct prognostic significance.[8]

4. If the patient's Wassermann fluctuates from week to week, as e. g. 0, 2+, 3+, 0, 4+, such variation is not necessarily a reflection on the laboratory, nor is it significant of any change in the patient. It may mean only that the serum in question contains minute quantities of reagin; comparatively slight but unavoidable variations in the delicacy and adjustment of the test would account entirely for the apparently discrepant results.

G. THE SO-CALLED BIOLOGIC FALSE POSITIVE WASSERMANN IN CONDITIONS OTHER THAN SYPHILIS

False positive reactions we have always with us. The great majority of such reactions are due to some technical error in the laboratory: inadvertent omission of complement; deterioration of complement under the conditions of the test; anticomplementary antigen, mislabelled specimens, etc. Fortunately, such mistakes are usually readily detectable by repeating the test before instituting treatment. Of much greater theoretical interest is the other type of false positive reaction, the true fixation of complement supposed to be obtained in conditions other than syphilis. In the early papers on the subject, all sorts of conditions and diseases, including pregnancy, leprosy, tuberculosis, pneumonia, scarlet fever, etc., were reputed to predispose regularly to nonspecific reactions. Gradually it came to be recognized that the bulk of these were not biologic false positive reactions, but were due either to the actual presence of syphilis, or to technical errors. Nevertheless, despite numerous papers in the literature to the contrary, the impression persists that nonspecific positive reactions are obtained frequently even in temperate climates, where yaws, recurrent fever, and trypanosomiasis can be more or less definitely excluded.

TABLE VI

DISEASE	NO. OF CASES	WASSERMANN NEGATIVE	ANALYSIS OF CASES WITH POSITIVE WASSERMANN			FALSE POSITIVE REACTION	DOUBTFUL WASSERMANN			FALSE DOUBTFUL REACTION
			TOTAL NO.	SYPHILIS PROVED	SYPHILIS DOUBTFUL		TOTAL NO.	SYPHILIS PROVED	SYPHILIS DOUBTFUL	
Pneumonia	87	73	12	8	3	1	2	2	0	0
Scarlet fever	14	14	0	0	0	0	0	0	0	0
Measles	13	13	0	0	0	0	0	0	0	0
Vincent's angina	5	3	1	1	0	0	1	1	0	0
Malaria	3	1	1	1	0	0	1	0	0	1
Acute infection	16	14	2	2	0	0	0	0	0	0
Unexplained fever	12	11	0	0	0	0	1	1	0	0
Tuberculosis	62	55	6	4	1	1	1	0	1	0
Alcoholism	38	34	3	1	2	0	1	0	1	0
Jaundice	62	49	11	9	2	0	2	0	1	1
Carcinoma pancreas	19	19	0	0	0	0	0	0	0	0
Carcinoma gastrointestinal tract	19	18	1	1	0	0	0	0	0	0
Carcinoma (miscellaneous)	15	13	2	2	0	0	0	0	0	0
Liver cirrhosis	13	11	2	2	0	0	0	0	0	0
Liver necrosis or atrophy	14	11	3	2	1	0	0	0	0	0
Pregnancy	38	35	2	1	1	0	1	0	1	0
Total	430	374	46	34	10	2	10	4	4	2

The statistics presented in Table VI give not the slightest evidence that such is the case. Four hundred and thirty patients were diagnosed as follows: scarlet fever (14), pneumonia (87), tuberculosis (62), jaundice (62), unexplained fever (12), acute infection (16), measles (13), carcinoma (53), alcoholism (38), Vincent's angina (5), liver affections (27), pregnancy (38), and malaria (3), all being conditions supposed to predispose to false positive reactions, had a Wassermann test during the course of the disease with an antigen containing at least 0.6 per cent sensitizing sterols. Of the total, 304 were four hour ice box tests, the remainder one-half hour water-bath tests. In the entire group there were 46 positive and 10 doubtful reactions. The difference between this incidence (10.7 per cent positive, 2.3 per cent doubtful) and the incidence of positive and doubtful reactions obtained in the general hospital and dispensary population (approximately 10 to 15 per cent) is hardly significant.

Moreover, of the 56 cases in which some type of positive result had been obtained, 38 presented either clinical or autopsy findings considered diagnostic of syphilis, a history of antecedent antisyphilitic treatment, or a positive serologic test before the onset of the disease under consideration. Of the remaining eighteen cases, there were 10 positive and 4 doubtful reactions in which the data available on the records were inadequate to allow any decision as to the presence or absence of syphilis. Inasmuch as about one-third of Wassermann-positive cases encountered in this dispensary present no evidence of syphilis, either clinical or in their history, (Turner 1930) it is probable that the majority, if not all, of these 12 individuals were similar cases of latent syphilis.

There remain only 2 positive and 2 doubtful reports in this entire group of 430 which can be properly termed false positive results. Of these, one positive reaction in a patient with pulmonary tuberculosis was obviously a technical error, and not due to the tuberculosis; for when the test was repeated, eight days later, it was entirely negative. The other positive result was in a case of pneumonia, Wassermann positive during the acute illness, and negative after the crisis. Of the two doubtful results, one was a case of malaria, and one a case of jaundice. Both were Wassermann doubtful during the acute illness, and negative thereafter. The rarity of these biologic false positive and doubtful results (one and two respectively in a total of 430 cases), in the very conditions which are supposed to predispose to their appearance raises serious doubt as to the existence of any causal relationship.

H. THE NATURE OF THE WASSERMANN REAGIN

The discovery that an alcoholic extract of normal tissue could be used as antigen in the Wassermann reaction seemed to prove definitely that the active substance in syphilitic serum was not an antibody to Sp. pallida. It is now generally believed that the Wassermann reaction is an anomalous phenomenon bearing not slightest resemblance to such true antigen-antibody reactions as bacterial agglutination, protein precipitation, or specific complement fixation. Despite this general impression, there is an increasing body of evidence that reagin may, after all, be a specific antibody produced in response to an antigenic stimulus.

To enumerate these points briefly:

1. The substance responsible for both the Wassermann and flocculation tests appears some time after the infection, the interval corresponding roughly to the time which elapses between the beginning of artificial immunization in the bacteria and the appearance of specific antibodies in the experimental animal. When the infected individual is treated with specific drugs, there is a fall in the Wassermann titer, corresponding to the disappearance of antibodies when injections of antigen (bacteria, red cells, protein) into the experimental animal are discontinued.

2. The physical and chemical properties of the active substance in syphilitic serum resemble those of true antibodies. Like them, it does not dialyze, is associated with the globulin fraction of serum, is only moderately affected by temperature less than 55° C., and is rapidly and irreversibly destroyed by heat $> 70°$ C. Like antibodies also, Wassermann substance is destroyed by formol.*

The physicochemical processes underlying the Wassermann and precipitation tests for syphilis are identical with those which determine specific aggregation and complement fixation. I have presented evidence elsewhere[9] to show that the active substance in syphilitic serum is deposited as a film of denatured protein around the colloidally dispersed lipoid particles in the antigen dilution. It is this film of reagin-globulin which is believed to adsorb complement (Wassermann reaction) and to cause the cohesion of similarly coated particles in the various flocculation tests (Kahn, Sachs, Georgi, Miller, Eagle, etc.) An exactly similar process determines the aggregation of bacteria, red cells, or dissolved protein by a specific

*Literature summarized in J. Exper. Med. **52**: 717, 1930.

antibody,[10] as well as the adsorption (fixation) of complement by the sensitized antigen particles.[11]

4. Finally, it has been possible to induce a positive Wassermann reaction of extremely high titer in rabbits as an antibody response to the injection of a lipoid antigen.[12]

Granted that these several lines of evidence do strongly suggest that reagin is an antibody, it must be admitted that there is the greatest doubt as to the nature of the antigenic stimulus which initiates its production. The obvious answer, that the antigen is the Spirocheta pallida, as originally suggested by Wassermann and recently again proposed by Klopstock,[13] seems to fall down on several grounds: (1) the fact that reagin combines with *normal* tissue lipoids; (2) the experimental finding that cultures of supposed Sp. pallida usually fail to induce a positive Wassermann reaction when injected into rabbits,[14] even though specific antibodies against the organisms are produced in high titer; and (3) the observation that cultures of Sp. pallida are not agglutinated by syphilitic sera[15] and cannot be used as antigen in the Wassermann reaction.[16] On the other hand, the suggestion of Sachs, Klopstock and Weil[17] that reagin is an antibody to autogenous lipoids, liberated at foci of infection and tissue destruction, seems to lack conviction. It is difficult to understand why only syphilis should cause such an intense autoantibody response, while infections in which the tissue destruction is just as marked fail to produce even traces of Wassermann reagin.

It is the feeling of the author, without as yet any experimental evidence, that the first conjecture is correct; and that reagin may after all be not an anomalous "colloidal state' but a true antibody to the Sp. pallida. The fact that normal tissue lipoids react with this antibody does not disprove this surmise, for Sp. pallida and mammalian tissue may well contain a common antigenic factor, exactly as guinea pig, kidneys, sheep red cells, and various bacteria contain a common lipoid, the heterophile antigen of Forssmann.

It is true that spirochetes cultured in artificial media usually fail to induce the formation of reagin in the experimental animal, and fail to react with the reagin of syphilitic sera. Nevertheless, the nonpathogenicity of these cultures for rabbits raises grave doubts as to the validity of their identification as Sp. pallida. This nonpathogenicity, and the aforementioned antigenic properties of the cultures, suggest indeed that such cultures may be either saprophytic contaminants, or degraded forms from which no conclusions as to the antigenic properties of Sp. pallida in vivo can be properly drawn.

The final decision as to whether reagin is an antibody to Sp. pallida must await the cultivation of a strain indubitably pathogenic for rabbits.

SUMMARY AND CONCLUSIONS

1. Progressive increase in the amount of sensitizing sterol in a Wassermann antigen causes a progressive increase in sensitivity, and does not result in the appearance of biologic false positive reactions. A supersensitized antigen (1 to 1½ per cent sterols) detects fully 50 per cent more syphilitic sera than are detected by a plain alcoholic extract. There is thus no need for the continued use of antigens containing either no sensitizer, or only 0.1 to 0.3 per cent.

2. It is unnecessary to use more than one good antigen. Different antigens

do not have selective affinity for particular clinical conditions; the number of sera they detect is determined solely by their sensitivity to reagin. When it is known which is the most sensitive of two or three antigens, no virtue attaches to the use of more than the one with maximum sensitivity.

3. The water-bath test (one-half hour at 37° C.) compares so unfavorably in sensitivity with an ice box test (four hours at 0° to 6° C. followed by one-half hour at 37° C.) as to make some type of cold incubation almost mandatory. Since the latter does not predispose to biologic false positive reactions, it is unnecessary duplication to perform both types of test.

4. *With a highly sensitized antigen,* the difference in sensitivity between an overnight ice box test, and a test involving four hours in the ice box, followed by one-half hour at 37° C., is too slight to justify the longer incubation as a routine procedure. This is particularly true in view of the greater number of anticomplementary serum reactions and the danger of complement deterioration in the eighteen hour test, and the fact that a highly sensitized antigen cannot be used in an overnight test because of its anticomplementary properties.

5. There is no evidence that acute infections, malignancy, tuberculosis, pregnancy, liver disease, or alcoholism regularly predispose to false positive Wassermann reactions.

6. It is unnecessary to carry out a cumbersome daily or weekly multiple titration of antigen (antigenicity; hemolytic properties; anticomplementary properties). Modern methods of preparation ensure uniform, highly sensitive antigens. One need only determine the anticomplementary titer of a particular lot of antigen, once and for all, and use it always in the same dilution, sufficiently removed from this anticomplementary zone to eliminate any possibility of error on that score.

7. The daily titration of complement is an illusory quantitation inasmuch as the cell suspension used as the measure of complement activity, is itself highly variable. It is suggested that the complement can be roughly standardized by using the pooled serum of 3 to 7 pigs, using it always twenty-four hours after bleeding, and in a constant arbitrary dilution of e. g., 1:12. One can then compensate for fluctuations in cell fragility by adjusting the amboceptor, using some arbitrary but fixed multiple of the minimal hemolytic quantity in the test.

8. The terms, 1+, 2+, 3+, and 4+ Wassermann reaction are misleading insofar as they cover only an insignificant fraction of the total variation in the reagin content of syphilitic sera. The term 4+ does not necessarily imply a "strongly" positive reaction, but applies to any serum with more than one unit of reagin, whether that serum contains 1 or 200 units. It is therefore recommended that Wassermann reactions be reported as negative (complete hemolysis), positive (no hemolysis), or doubtful (incomplete hemolysis). If a truly quantitative reaction is desired, it is necessary to carry out a test on serial dilutions of serum (1:1, 1:2, 1:4, 1:8, 1:16, 1:32, etc.).

9. Contrary to the accepted view that the Wassermann reaction is an anomalous and unique biologic test, there is a considerable body of evidence that the intimate physicochemical processes involved in the reaction are exactly similar to those which determine specific antigen-antibody reactions: that syphilitic reagin may after all, be an antibody to products of infection. The nature of the antigenic sub-

stance responsible for its appearance, whether Sp. pallida, as originally believed by Wassermann, or autogenous lipoids, remains in doubt.

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A METHOD FOR STUDYING THE ACTIVITY OF INTACT SKELETAL MUSCLE*

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THE method herein described was employed by Leese, Hines, and Jordan[1] in studying the influence of fasting upon the activity of skeletal muscle. We believe that it has several distinct advantages over the methods ordinarily employed in this type of work. By this method one is able to study the activity of individual muscles with a minimum of disturbance to the natural physiologic conditions of the muscle.

A survey of the experimental work of previous investigators showed that in general either one of two procedures have been employed for the study of activity of individual muscles. The earlier and more frequently used method was that of attaching the isolated muscle to some form of recording apparatus. Although a great amount of information concerning muscular activity has been compiled by means of this method, its limitations for the study of fatigue are well recognized. The absence of circulation and nervous contact is sufficient to greatly alter the physiologic behavior. Realizing these difficulties, numerous workers have devised methods whereby muscular response may be recorded under conditions permitting an intact circulation with or without complete nervous contact. Cannon and Nice,[2] Gruber,[3, 4, 5, 6] Gruber and Fellows,[7] Gruber and Kretschmer,[8] Miley,[9] Gans and Miley,[10] Gans and Hoskins,[11] and numerous other investigators have used the cut tendon method. This method involves the sectioning of the tendon of an intact muscle and the tying of the muscle by means of a string to the apparatus in order that the muscular response may be recorded. In some instances undue stretching was prevented and in others little attention was paid to it. Experiments with and without the cut tendon suggested to the authors that the technic of tendon cutting usually resulted in disturbances difficult to control even though efforts were made to maintain conditions as normal as possible.

In order to prepare fatigued skeletal muscle for glycogen analyses, a method was designed to study muscular fatigue under conditions more ideal due to the absence of any operative treatment on the muscle used or on its attachments. Those disturbances to circulation and nervous behavior caused by the stretching or manipulation of the muscle were prevented by retaining the natural attachments of the muscle. The after-loading problem was eliminated in the same manner.

Procedure.—The animals used for these experiments were adult rats selected from the general laboratory stock. Sodium amytal in doses of 6 mg. per 100 gm. of body weight was administered by the intraperitoneal route. This amount was sufficient to produce a satisfactory degree of anesthesia. The extent of the superficial

*From Department of Physiology and The Child Welfare Station, State University of Iowa. Received for publication, July 20, 1932.

reflexes was used as a criterion of the depth of anesthesia. Additional amounts of anesthesia were injected from time to time, either by means of the automatic apparatus built for this purpose or by a Luer syringe.

Break induction shocks were delivered directly to the gastrocnemius group of one limb of the rat at the rate of 2 to 3 per second. The contractions of the gastrocnemius group were recorded on a slow-moving extension kymograph. The stimulation was continued until no appreciable amount of shortening could be detected. The animal was then removed from the apparatus and the muscles excised and weighed. In some instances the tendon achilles was cut and tied to the recording device. After-loading adjustments were carefully made. In other experiments the recording apparatus was attached to the toes and the tendon left intact. Corrections were made for the calibration of the spring and magnification due to leverage.

Description of Apparatus.—The stimulating unit permitted the sending of break stimuli to the tissue in uniform manner for as many hours as desired. It needed no attention except an occasional brushing of the mercury with a damp camel's hair brush. The unit was operated by a quarter horse power Westinghouse motor governed by a rheostat. The gearing was so arranged that the motor revolved sixty times to one revolution of the drum carrying the stimulating contacts. Such an arrangement should minimize the effect of slight variations in the house current. By means of a rheostat, any desired rate of stimulation could be effected between one stimulus every two seconds to one hundred stimulations per second.

The recording unit consisted of a Harvard recording lever, an adjustable calibrated spring, and a kymograph. The resistance of the Harvard lever and two pulleys was added to the load. The kymograph moved from 18 to 24 inches per hour and permitted about 1000 contractions to be recorded in a space of from 6 to 8 cm. The paper was adjusted so it moved in a slight spiral fashion. Such an arrangement permitted freedom from handling for as long as 20 or more hours. The rat was fixed to the apparatus by means of a modified femur clamp adjusted to the tibia. A slit ½ of an inch in length was made in the skin over the tendon and over the gastrocnemius, in order that the glass-shielded electrodes could be more easily pushed in place.

Fig. 1 indicates the mechanics of the stimulating unit. The contact points *A*, *B*, and *C*, which were soldered to the drum, made contact with the mercury cups on the board, *K*. The couplers *A*, *B*, and *C*, represent the means of current departure from contacts made by the contact points of like letter. Each contact point was made with an adjustable coupler, into which was fastened a steel needle of the best quality. Steel was used because of the relative absence of alloy formation with mercury. Contact point, *D*, was similar to the others and operated in the mercury cup, *L*. The explanation for the various electrical contacts will be given in the discussion of Fig. 2.

The drum to which the contact points *A*, *B*, and *C*, were fastened had a diameter of 160 mm., and was propelled by a belt, *F*, which in turn operated on a pulley shaft having a diameter of 10 mm. The shaft was the axis of a 160 mm. diameter pulley upon which was placed a second belt to the motor shaft, the diameter of which was 5 mm.

A trip key, *G*, was fastened to the drum in such a manner that it turned the cyclometer, *H*. By this arrangement the total number of stimulations was automatically recorded.

The rubber tubes, *E*, were inserted into the board, *K*, and passed to a bottle, *M*. They were for the purpose of permitting the collection of mercury which was frequently brushed from the mercury cups. The mercury was cleaned and used again.

Fig. 2 indicates the arrangement of the mercury cups on board, *K*, of Fig. 1. Drawing *X* represents the arrangement of the mercury cups. The cups in the outer rows had a diameter of 1 cm. Each cup passed completely through the board and

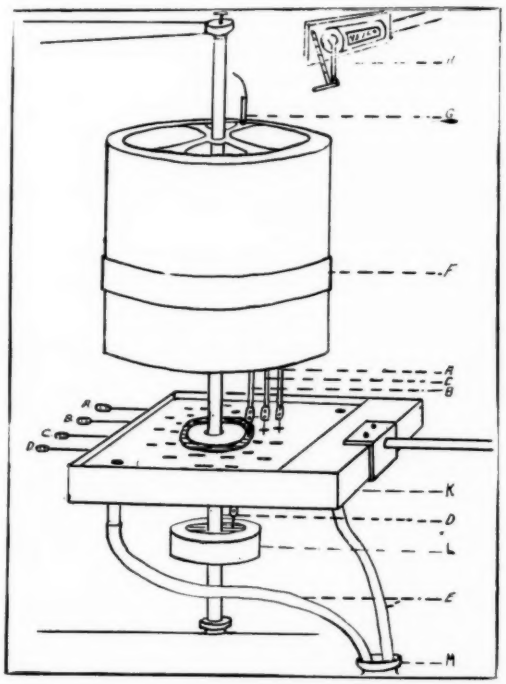

Fig. 1.—The stimulating unit. *A*, *B*, *C*, *D*, contacts and their electrodes; *E*, rubber tubes; *F*, belt; *G*, cyclometer key; *H*, cyclometer; *K*, contact board; *L*, mercury cup; *M*, mercury bottle.

was plugged with a cork stopper containing a steel electrode as indicated in drawing *Y* of Fig. 2. This arrangement made it possible to repair the connection to any cup, if necessary, without any disturbance to the rest of the board.

Mercury cup, *C*, did not pass through the board, but was a moat 1 cm. wide and 5 mm. deep. It was also provided with a steel electrode from the under side of the board.

Drawing *Y* represents the opposite side of the board indicated in *X*, and presents the wiring of the mercury cups on board *K*.

It will be noted that lead, *A*, was a composite of the 16 outer cups which were in the primary circuit. Lead *B*, was a composite of the inner row of 16 cups which were intermittent short circuiting keys in contact with one of the secondary poles of the inductorium. Lead *C* was in contact with the circular moat, *C*, of diagram

X and was a constant short circuiting key to the other pole of the secondary of the inductorium. By means of *B* and *C* the "make" current was automatically shorted out. The nature of the intermittent short circuiting keys, *B*, permitted the "short" to be broken in time to allow the "break" current to go through undisturbed.

The revolving drum, *D*, in diagram *X* shows the manner by which the shorting of the "make" was effected. Contact was made with *B* and *C* together before the primary circuit through *A* was closed. With the closing of *A* the "make" was shunted across the short circuit on the secondary and consequently did not reach the muscle. Contact with the intermittent short circuiting keys, *B*, was broken before *A* was broken. Therefore the break current went out over the secondary and to the muscle.

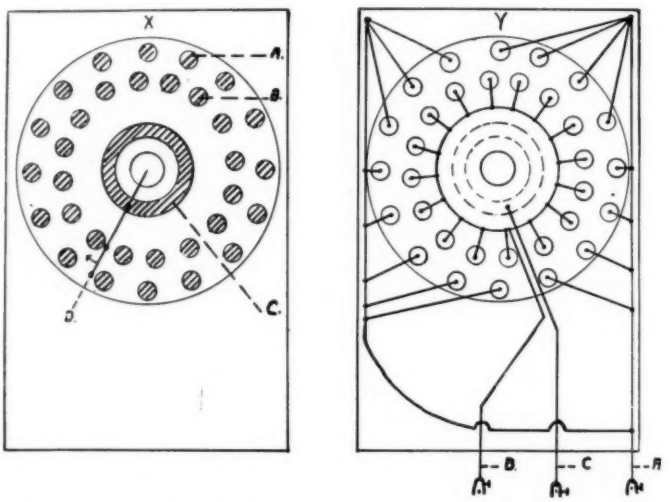

Fig. 2.—*X*, Top of contact board; A, primary contact; B, intermittent short circuiting contact; C, constant short circuiting contact; D, drum carrying contact points. *Y*, Bottom of contact board; *A*, primary electrode; *B*, intermittent short circuiting electrode; *C*, constant short circuiting electrode.

Fig. 3 represents the manner by which the animal was maintained for activity and shows the electrodes in place.

The inset shows the electrode in detail. *F*, was the glass tubing housing the metal electrode. The glass housing was drawn in a tapering manner, *C*, over the entire metallic portion of the electrode. Such an arrangement permitted the adjustment of the tip, *A*, below the skin surface without shorting on the skin or subcutaneous tissue. The diameter of the housing was one-fourth of an inch and possessed a sufficiently large bore to permit the adjustment of the metallic electrode. After adjusting the metal the housing was heated and drawn to form a thin insulating covering over the metal.

The number 24 copper wire, *E*, was soldered at *D*, to a steel needle, *B*. The needle protruded from the housing at *A* for about one-eighth of an inch. The total length of the electrode was about 6 inches.

The electrodes were held in place by a twisted rubber band, *H*, which permitted unhampered movement of the electrodes. The rubber band, *H*, was in turn

supported by an insulated fork, *G*, which was fastened to an adjustable stand. When the electrodes were properly adjusted they appeared as indicated by *I* of Fig. 3.

Fig. 4 indicates the arrangement of the recording unit. The string, *J*, which was attached to the foot by a pin hook passed over pulley 1, and was fastened to the recording lever 1 cm. from the fulcrum. A continuation of the string descended from the recording lever and passed over pulley 2, and was attached to a calibrated spring, *A*. The calibration was equal to 1 gram per mm. The spring was initially set by the fixation of set screw, *D*, and the adjustment of the clamp *X*, so that the muscle was always after-loaded with a load amounting to exactly one-half of his own weight. The set screw, *D*, was then released.

It will be noted that the fixation of all parts of the recording apparatus em-

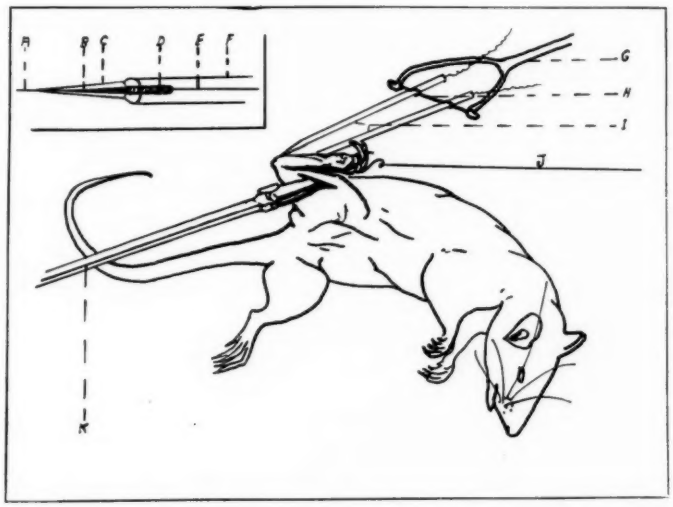

Fig. 3.—Adjustment of animal to apparatus. *A*, Steel electrode point; *B*, steel needle; *C*, glass insulating shaft; *D*, soldered joint between copper wire and steel electrode; *E*, copper wire; *F*, glass shaft; *G*, electrode support; *H*, rubber band; *I*, electrodes in contact with animal; *J*, string between animal's foot and recording unit; *K*, clamp holding tibia.

phasized rigidity. The shaft, *C*, upon which the entire mounting took place was a three-quarter inch bore steel pipe anchored to cement ceiling beams and to the cement floor.

The recording lever measured 15 cm. from the fulcrum to the tip of the paper writing point, with the string set 1 cm. from the fulcrum. The smoked recording paper was operated by two Harvard drums accommodated with a "ticker" for slow speeds. The length of the paper was measured so as to permit one complete revolution in about five hours.

The adjustment of the animal to the apparatus is shown in Fig. 3. When the electrodes were properly placed one was thrust into the gastrocnemius muscle and the other was inserted into the tendon Achilles. The leg was held in place by the clamp, *K*, which was attached directly to the tibia. The only operative work upon the animal occurred here and amounted to a skin slit about one-half inch in length and the separation of the subcutaneous fascia as well as the bone fascia, so that the

tibia was readily accessible. Care was taken not to injure any blood vessel. Care was also used to insure the absence of any other structure in the clamp except bone. The clamp, *K*, was rigidly fastened to the work table and had absolutely no flexibility or vibration.

It is extremely essential to note that the tendon was not cut and that the set-up was in the nature of an ergographic technic so arranged that the after-loading of the muscle was taken care of by the natural anatomical arrangement of the bony levers. Such an arrangement was emphasized because of its physiologic naturalness.

The string, *J*, was fastened to the foot by a pin-hook which was always thrust

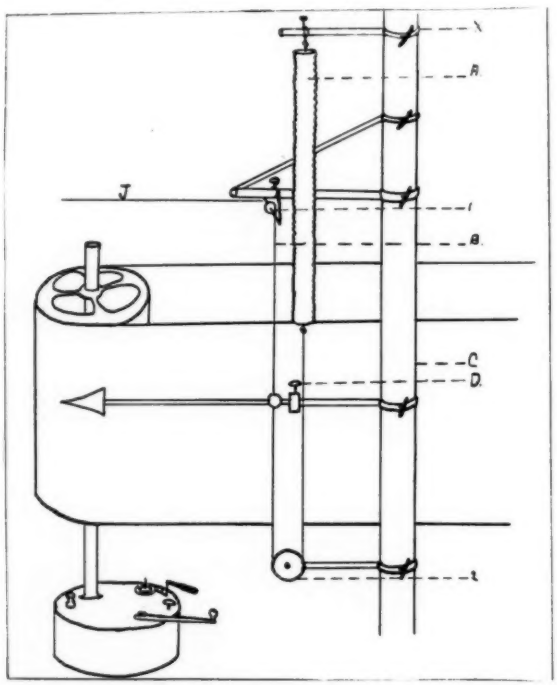

Fig. 4.—Recording unit: *A*, spring; *B*, string attached to recording unit; *C*, ¾-inch bore pipe; *D*, after loading screw; *J*, string as it passes from animal to apparatus; *X*, adjustable clamp; *1*, pulley; *2*, pulley.

TABLE I

A SUMMARY OF THE KILOGRAM METERS OF WORK DONE PER GRAM OF MUSCLE BY THE AUTHOR'S AND THE CUT TENDON METHODS

	AUTHOR'S METHOD	CUT TENDON METHOD
	1.38	0.0262
	5.30	0.0290
	3.28	0.0290
	1.72	0.0631
	2.70	0.0285
	2.80	0.0647
	2.55	0.0527
	0.71	
Average	2.555	0.0419

through the middle phalangeal joints of the toes. The string was the best quality 20 pound test silk fish line. It was suspended from the ceiling with a 20 pound weight for ninety-six hours before use in order to take out all of the "stretch" which it might have had.

CONCLUSIONS

A method is described for studying the activity of the intact skeletal muscle of the anesthetized rat which permits much more work to be done by the muscle than when the usual methods are employed. This method permits one to retain the natural attachments of the muscle and thereby minimize disturbances caused by such conditions as over-stretching, drying, temperature changes and interferences with the circulation and nerve control. A convenient automatic stimulating unit is described which permits a wide range in the choice of intensity and frequency of the stimulus. Most of the material required can be assembled from that usually found in a physiologic laboratory.

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A HISTOCHEMICAL METHOD FOR THE DETECTION OF CHOLESTEROL*

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PRACTICALLY all of the numerous sterol color reactions are based on the use of such anhydrous reagents as arsenic trichloride, acetic anhydride, or sulphuric acid. According to Rosenheim[1] the chromogenic properties of sterols in the presence of strong acids is due to the formation of carbonium salts which are colored. The colors produced are transitory and are discharged in the presence of moisture. Either the Salkowski or Liebermann-Burchard reaction in which concentrated sulphuric acid is used, is made the basis for most histochemical methods for the detection of cholesterol. They are all unsatisfactory, as the rapid play of colors is difficult to follow under a microscope, and the strong acid destroys the section within a very short time.

The physical characteristics of cholesterol preclude the possibility of the finding of a dye which will stain cholesterol within the cell. Fat soluble dyes are surprisingly few in number and unsatisfactory for the purpose. Unfortunately, stable chemical compounds of cholesterol which could be formed, are practically all colorless. Steinle and Kahlenberg[2] describe a dark brown substance formed by reaction with antimony pentachloride. This is an addition product of cholesterol and antimony pentachloride having the formula $C_{27}H_{46}O.SbCl_5$. The reagent used in the following method consists of a 25 per cent antimony pentachloride solution in chloroform, to which a few crystals of antimony trichloride have been added in order to remove any free chlorine.

The procedure is as follows: The section is transferred to a slide, and blotted carefully three times with filter paper. It should not be absolutely dry. Treat with 1 or 2 drops antimony pentachloride reagent. A dark brown color develops instantly wherever the reagent comes in contact with cholesterol or its esters. Allow the excess reagent to evaporate. The white powdery substance, which sometimes forms, disappears on blowing the breath over the slide. Wash with 1 or 2 drops chloroform, and mount in glycerol. Unsaturated fatty acids are stained a light orange, and may thus be differentiated from the dark brown of the cholesterol.

In very thin sections containing only traces of cholesterol, the fumes of antimony pentachloride may be used more satisfactorily than the chloroform reagent. A small glass rod is dipped in antimony pentachloride, and then held over the section until the pentachloride fumes have thoroughly penetrated the tissue. This obviates the necessity of using chloroform which may remove the cholesterol. After the brown colored cholesterol compound has formed, the section may be washed with a drop or two of chloroform, and the washings removed from the slide by means of filter paper.

*From the Biochemical Laboratory of the Metropolitan Life Insurance Co.
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The brown compound of cholesterol is not readily soluble in choloroform. It does, however, dissolve slowly, yielding a deep purple color. If the section is mounted in chloroform, the deep brown slowly takes on a purplish hue, and thereby furnishes a further differentiation between cholesterol and unsaturated fats. When mounted in glycerol, a green brown color develops. There is no evidence of the color fading, even when the slide is exposed to sunlight for days.

The above procedure furnishes a rapid method for the detection of cholesterol in the various regions of the section. Unfortunately, it does not demonstrate cholesterol within the cell. The cell wall is either ruptured in the process, or there is a gradual diffusion of the coloring matter within the cell.

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THE EXPRESSION OF HEMOGLOBIN CONCENTRATIONS ON A MOLAR BASIS*

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THE adoption of a uniform method for the expression of hemoglobin values, in terms of grams of this pigment per hundred cubic centimeters of blood, may be considered an advance over the arbitrary "percentage of normal" values commonly employed. Through the medium of gram percentage, we may offset the differing standards employed by the hemoglobinometers in common use, and, provided these have been accurately standardized, we may correlate the results with different instruments and in different laboratories. The disadvantage of the "normal percentage" reading has been pointed out.[1]

Like all chemical substances present in blood, hemoglobin concentrations have a normal range rather than a normal fixed point, this range varying with various environmental factors. Whereas hemoglobin determinations in themselves are probably of more value than an isolated erythrocyte count, and repeated hemoglobin determinations, performed under identical conditions of blood dilution may be useful in following the course of hemorrhage or treatment, we have only one index as to the identity of blood dilution; a simultaneous red count.

The classification of anemias into macrocytic and hypochromatic varieties places more stress than ever on an accurate delineation of the ratio of pigment to corpuscular stroma. This is possible only where we have the hemoglobin concentration and the erythrocyte count at hand and much discrepancy between investigators might be avoided if their "normal" hemoglobin values were recalculated to an erythrocyte count of five millions. This is, in effect, a color index calculation. Any marked variation in the color index is significant of erythroblastic pathology;

*From the Laboratory of Clinical Pathology of the Chicago Free Dispensary and the Department of Physiology of the Chicago Medical School.
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either the lack of adequate substances essential in mature corpuscular synthesis (high color index) or those necessary for pigment formation (low color index).

A general recognition of the utility of the color index is exemplified by the fact that the arbitrary "100 per cent" has persisted. Even in the newer forms of hemoglobinometers, there is designated some particular concentration of the pigment to correspond with 100 per cent (excepting Haden's hemoglobinometer). There has been another, perhaps more potent, reason for the retention of this latter figure. For years, in our courses in physiology and biochemistry we have tried to engender into our students, the propriety and necessity for accurate hemoglobin designations, only to see these same students, during their clinical years and interneships, fall back into the fallacies of 100 per cent on no scale whatsoever.

For these reasons we have adopted the policy of allowing the 100 per cent to become 100 decimillimolar. By that maneuver "100" becomes a ponderable figure. Subsequently Avery and Hastings[3] (1931) pointed out the facility of expressing concentrations of other blood constituents on a molar basis. In the case of hemoglobin there is particular legitimacy for such expression. Where the physiologic rôle of a substance is as well understood as is that of hemoglobin, molecular designations give an immediate insight into the concentrations of those substances with which hemoglobin is identified. For instance, blood containing 100 decimillimolar hemoglobin is 100 decimillimolar in iron, oxygen capacity and hematin as well (Table I). It remains to be seen whether the decimillimolar basis of expression is equal in exactitude to the gram percentage basis and whether it is a convenient figure for the calculation of color indices of human blood.

TABLE I

EQUIVALENTS OF 100 DECIMILLIMOLAR HEMOGLOBIN IN CONCENTRATIONS OF

HEMOGLOBIN		HEMATIN		IRON		O_2 OR CO CAPACITY	
GRAMS %	ARBITRARY %	GRAMS %	MOLARITY IN DECIMILLIMOLS PER LITER	GRAMS %	MOLARITY IN DECIMILLIMOLS PER LITER	VOLUMES %	MOLARITY IN DECIMILLIMOLS PER LITER
16.67	Dare (old) 121.1 Fleischl 105.5 Talquist 105.5 Dare (new) 104.9 Barnard 100.0 Newcomer 98.6 Sahli 96.3	0.6396	100	0.05546	100	22.4	100

Hemoglobin differs from most proteins in providing excellent criteria for the determination of its molecular weight. Of these criteria we have:

1. The oxygen or carbon monoxide combining power. Since the ratio is always one mole of either of these gases to one mole of hemoglobin we may base a determination of molecular equivalency on the amount of oxygen or carbon monoxide chemically combined.

2. One molecule of hemoglobin can contain not less than one molecule of iron. From its iron content, Barcroft and Hill have calculated a minimum molecular weight of 16,669.

3. Osmotic pressure determinations by Adair[2] (1929), sedimentation velocity studies by Svedberg and Nichols[13] (1927) and determinations of diffusion constants by Northrup and Anson[12] (1929) have led to a calculation of the actual molecular weight of hemoglobin as being four times that reported by Hüfner and Gausser[11] (1907) from their osmometer studies. There is apparently an association of four prosthetic radicals with globin in the formation of the molecule, but the equivalent weight, for practical purposes may be taken as 16,670.

4. Conant[8] (1923) and Conant and Fieser[9] (1924) have shown that hemoglobin may be titrated electrometrically with potassium ferricyanide. One iron equivalent of hemoglobin will reduce one mole of the oxidizing agent. The oxidation of hemoglobin to cyanmethemoglobin is easily followed potentiometrically and in this laboratory the method has been applied to the estimation of hemoglobin in blood (Fig. 1).

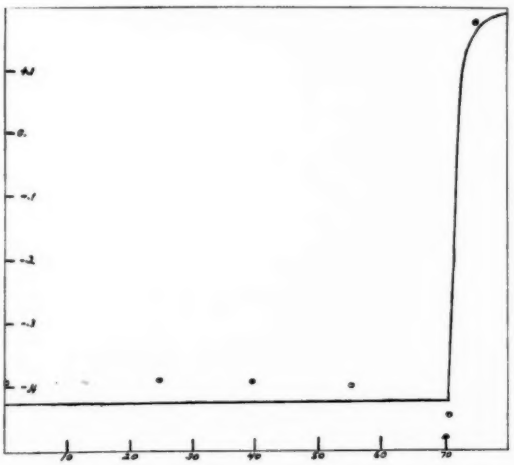

Fig. 1.—Electrometric titration of hemoglobin in blood. One cubic centimeter of blood containing 70 decimillimolar hemoglobin was centrifuged to throw down the corpuscles. The serum was discarded and the cells were hemolyzed with 10 c.c. 0.1 per cent NaCN solution. This was transferred to an electrometric titration vessel, 10 c.c. of ethyl ether was added and the sample titrated with 100 decimillimolar $K_3Fe(CN)_6$ solution. Stirring was effected by a nitrogen inlet tube.

The abcissa represents decimillimols of ferricyanide X 10^{-3} added, ordinates are potentials against a bright platinum electrode on the saturated KCl-calomel scale.

5. The hematin content of hemoglobin, with our present exact knowledge of the constitution of hematin, may be used as a basis for estimation of the molecular equivalency of the latter.

In place of these five criteria for the molecular concentration of hemoglobin solutions, we have only one ultimate criterion for the gram percentage of such solutions, this depending on an accurate determination of the dry weight of the substance. In such a procedure there is always the question of (1) inadequate drying, (2) too extensive drying with decomposition of the molecule, (3) the identity of the globin moiety, and (4) admixture with other substances. Hemoglobin is a hydrated protein and one wonders whether it will yield this water, chemically combined or physically associated, without dissolution. The globin fraction makes up 96 per cent of the molecule, and its constancy of composition is open to doubt, Finally, it is certain that those preparations of hemoglobin which were used to de-

termine some of the physical and chemical properties of the pigment, were not isoelectric, but actually sodium and potassium hemoglobinates.

On the basis of the work of Butterfield, who gives the percentage of iron in human hemoglobin as being 0.0334 per cent and correcting for the amount of potassium which would have been associated with preparations crystallized at P_H 7.4, we may take the concentration of a 100 decimillimolar hemoglobin solution as being 16.67 per cent. This is a convenient figure, since it is an aliquot of 100 and should it be taken as the 100 of a hemoglobinometer scale (Barnard,[5] 1932), the conversion of grams to decimillimols or vice versa is accomplished simply by multiplying or dividing by six.

TABLE II

HEMOGLOBIN ARBITRARY NORMALS FOR COLOR INDEX DETERMINATION RECALCULATED TO A DECIMILLIMOLAR BASIS

(100 DECIMILLIMOLAR = 16.67 GRAMS OF HEMOGLOBIN PER HUNDRED C.C. OF BLOOD)

HEMOGLOBINOMETER	STANDARD	METHOD	DECIMILLIMOLAR	
Fleischl-Miescher	Oxyhemoglobin		94.8	
Tallquist	Oxyhemoglobin		94.8	
Dare (new)	Oxyhemoglobin		96.0	
Barnard	Cyanhematin		100.0	
Newcomer	Hematin		101.6	
Sahli	Hematin		103.8	
			Average	98.5
Authorities:				
Haden		O_2 capacity	93.6	
Barnard and Woolley[1]		Cyanhematin	97.9	
Wintrobe[2]		Hematin	96.4	
			Average	96.0

[1]Unpublished results.
[2]Calculated from data on mean corpuscular concentration and mean corpuscular volume (1931).

We must next inquire into the proximity of the 100 decimillimolar figure to the actual hemoglobin content of those samples of human blood which average five million red cells per cubic millimeter. Here there is recourse to data in the literature as well as to the "100" calibrations of those hemoglobinometers which are used in the calculation of color indices. It is quite obvious from Table II, that the 100 decimillimolar value, taken as the hemoglobin concentration to correspond to 100 per cent of erythrocytes, practically coincides with other values in use and for that reason its universal adoption is advocated.

SUMMARY AND CONCLUSIONS

It is suggested that the arbitrary 100 per cent scale of hemoglobin designation, as ordinarily employed for the calculation of color indices, be replaced by the ponderable decimillimolar scale. One hundred decimillimolar corresponds to 16.67 gm. per cent of hemoglobin and is approximately equal to the average concentration of the pigment in normal adult human blood averaging five million red cells per cubic millimeter.

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AN INSTRUMENT FOR THE RAPID PREPARATION OF POTATO CYLINDERS FOR DIAGNOSTIC CULTURE PURPOSES*

Stuart Germain, Denver, Colo.

THE use of potato cylinders for culture medium in bacteriologic practice dates back almost to the inception of bacteriology, yet this medium had not, until recently, proved of sufficient public health interest that the preparation of large numbers of cylinders from potatoes occupied an importance beyond that which could not be fulfilled by the ordinary laboratory equipment, such as a cork borer, and a small knife. Within the past few years, however, the potato cylinder has occupied a more important practical aspect in serving as the basis for a simple and rapid culture method for the diagnosis of tuberculosis in cases negative microscopically by the smear test.† Numerous requests for information regarding a suitable potato cylinder cutter prompted this brief description of an instrument devised and prepared by Mr. Sam Pollak for use at the National Jewish Hospital at Denver about five years ago.

The instrument as shown in Fig. 1 is prepared from a piece of brass tubing six inches long and five-eighths of an inch in diameter. This size tubing cuts potato

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*From the Research Department, National Jewish Hospital at Denver.
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†Corper, H. J.: The Certified Diagnosis of Tuberculosis, Practical Evaluation of a New Method for Cultivating Tubercle Bacilli for Diagnostic Purposes, J. A. M. A. **91**: 371, 1928.
Corper, H. J., and Uyei, Nao: The Cultivation of Tubercle Bacilli, J. Lab. & Clin Med. **13**: 469, 1928; Further Observations With a New Method for Cultivating Tubercle Bacilli, Ibid. **14**: 393, 1929; A Simple Glycerol Water Crystal Violet Potato Cylinder Medium for Diagnostic Cultures of Tubercle Bacilli, Arch. Path. & Lab. Med. **7**: 835, 1929.

cylinders suitable for the commonly used six by five-eighths inch bacteriologic test tubes. If desired the cutter can be made of any size brass tubing suited to cutting potato cylinders to be used in any size bacteriologic test tubes. The lower cutting edge is sharpened similar to that of the usual cork borer and remains in tubular form for about one and one-quarter inches after which the tubing is cut to one-half diameter for about three inches gradually tapering again at the top to the full diameter of the brass tube where the inserted hard wood handle cut on a slant and fastened with a small screw to the brass tube acts as an ejector for the cut potato

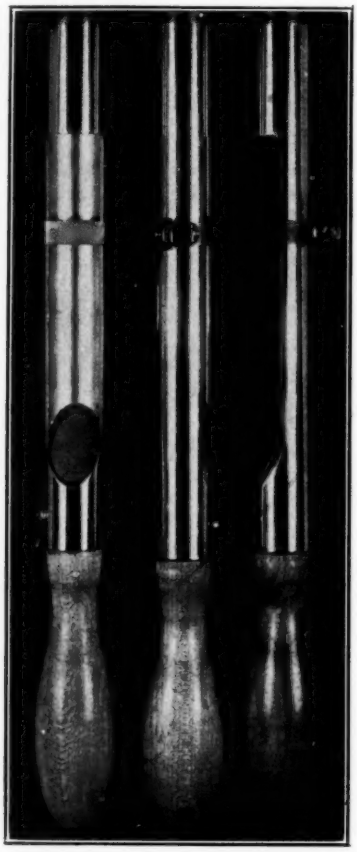

Fig. 1.

cylinders. About two inches above the cutting bottom edge a spring steel blade is bolted to the half diameter brass tubing for the purpose of halving the potato cylinders as they move up in the process of cutting. The nut and bolt clamp on the back of the spring steel cutter serves the purpose of adjusting this blade at any level desired by the technician. After the instrument has been suitably prepared the brass tubing is finally nickel plated to prevent corrosion and the hardwood handle is shellaced or varnished if desired. Properly cleaned and peeled potatoes after being suitably sized by cutting parallel pieces from opposite ends can be divided into halved cylinders about as quickly with this apparatus as the operator

can raise and lower his hand. The potato for cutting is placed on a clean board covered with a suitable rubber pad which serves to protect the cutting edge of the potato cylinder cutter. Any mechanic with a few tools and the necessary material can readily prepare a number of these cutters within a short time. If properly handled and cleaned after each use they may serve for a period of several years in our experience.

A GRAPHIC METHOD OF DETERMINING CERTAIN NUMERICAL FACTORS IN METABOLISM*

WALTER GOLDFARB, B.S., NEW HAVEN, CONN.

THE principle on which this method has been based is the same as that found in the papers of DuBois (1924) and Michaelis (1924). The determination of the numerical factors in metabolism in Michaelis' paper involves the use of two charts.

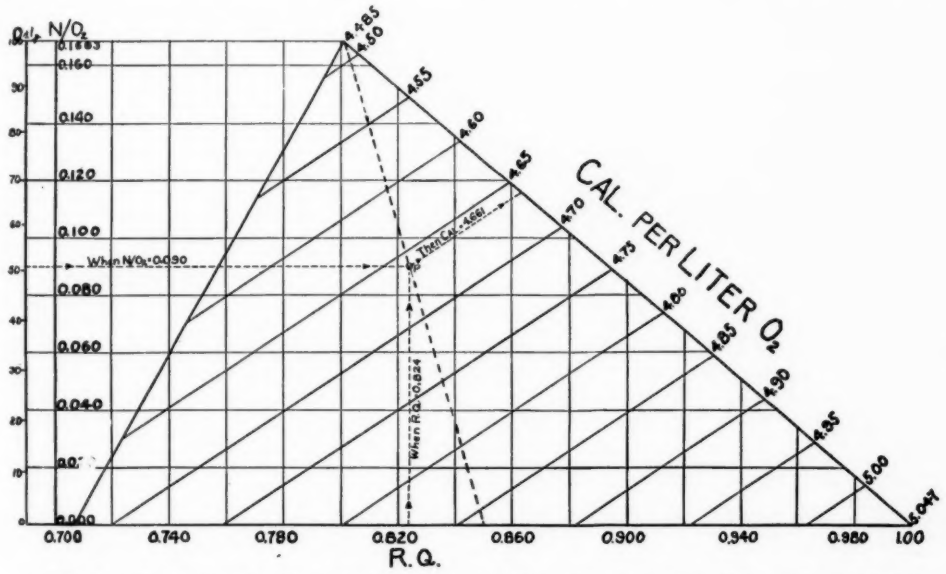

Fig. 1.—Chart showing the calorific value of a liter of oxygen according to the total R.Q. and the relationship of urinary nitrogen and total oxygen consumption. (Modified from Michaelis, Fig. 2.)

We have found it possible to obtain the same factors more readily using only the simpler of the two charts (Fig. 1).

If the urinary nitrogen (in grams), the total oxygen consumption (in liters), and the total respiratory quotient are known, we can obtain all the factors as follows: The point P is obtained on the chart from the total respiratory quotient and the ratio $\frac{\text{urinary-nitrogen (in grams)}}{\text{oxygen consumption (in liters)}}$. The caloric value of one liter of oxygen

*From the Department of Physiology, School of Medicine, Yale University.
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used for the mixture of foodstuffs is obtained by projecting P on the right side of the triangle parallel to the solid oblique lines drawn through the triangle. The percentage of the oxygen used for protein is read off the vertical scale on the left of the triangle. A line drawn through the upper vertex of the triangle and P will intersect the respiratory quotient scale at the base at a point equal to the nonprotein respiratory quotient. Now

$$(1)\ \%\ \text{calories from protein} = \frac{\%\ O_2\ \text{for protein} \times 4.485}{\text{calories per liter } O_2}$$

$$(2)\ \%\ \text{calories from carbohydrate} = \frac{(100 - \%\ \text{cal. from protein})\ (\%\ \text{cal. from carbohydrate at nonprot. R.Q.})}{100}$$

$$(3)\ \%\ \text{calories from Fat} = 100 - (1+2)$$

The only change made in Fig. 2 of Michaelis' paper is the line drawn through P and the upper vertex of the triangle. The constants used are the same.

For purposes of demonstration the same example used in Michaelis' paper is chosen. The ratio $\frac{N}{O_2}$ has been taken as 0.090, and the R.Q. at 0.824.

$$\%\ O_2\ \text{for Protein} = 53.5$$
$$\text{Calories per liter } O_2 = 4.661$$
$$\%\ \text{cal. protein} = \frac{53.5 \times 4.485}{4.661} = 51.5$$
$$\text{nonprotein R.Q.} = 0.850$$
$$\%\ \text{cal. carbohydrate} = \frac{48.5}{100}\ (50.7^*) = 24.6$$
$$\%\ \text{cal. fat} = 100 - (24.6 + 51.5) = 23.9$$

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*Constants at R.Q. 0.850 from Lusk, *Science of Nutrition*, fourth edition, 1928, p. 65.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

VINCENT'S ORGANISMS, Pathogenicity of the Fusiform Bacillus and Spirillum of Plaut-Vincent, Lichtenberg, H. H., Werner, M., and Lueck, E. V. J. A. M. A. 100: 708, 1933.

Attempts at producing lesions in any way similar to those commonly attributed to the action of the Plaut-Vincent organisms by injecting pure cultures of fusiform bacilli into areas of traumatized tissue in guinea pigs were unsuccessful.

The fusospirochetal organisms were found in 45.4 per cent of tonsils removed from 108 children.

In the same children these organisms were found in 91 per cent of the membranes that formed over the tonsillar beds after tonsillectomy, and usually in greater numbers than in the tonsils themselves.

The organisms were found constantly in smears of the membranes that formed over traumatic ulcers produced in the mouths of guinea pigs.

Neither the injection nor the local application of sulpharsphenamine hindered the appearance of these organisms in the lesions in the mouth of guinea pigs or hastened the healing of the lesions.

Sixteen consecutive cases of severe ulcerative stomatitis in children all healed in some four to seven days without treatment. This compares favorably with the reports of cases treated with various drugs and other forms of treatment.

The value of diagnostic smears for Vincent's organisms as a means of establishing a pathogenic relationship of these organisms to a suspected lesion is questioned.

SPINAL FLUIDS, Cell Count and Encapsulation of Brain Abscess, Woltman, H. W. J. A. M. A. 100: 720, 1933.

Abscess of the brain presents a highly varied clinical panorama that cannot be reduced to a simple formula. Each case is a law unto itself. The problem calls not only for a diagnosis but also for decision as to when the abscess shall be drained. Awaiting the optimal time means a better capsule and a liquefied interior, and consequently better drainage; it means less virulent organisms and greater immunity, and hence less danger to the patient.

The risk of performing spinal puncture in cases of abscess has probably been overrated, and thus physicians have been deprived of information that might be helpful.

After invasion of the brain has taken place and the formation of an abscess gets under way, the number of neutrophils in the spinal fluid becomes absolutely and relatively reduced.

The persistence or reappearance of neutrophiles suggests that encapsulation is not progressing favorably.

An appreciable number of neutrophils may indicate extension of the abscess or close proximity of the abscess to the ventricle.

A predominance of neutrophils in the spinal fluid was seen in cases in which operation was performed unnecessarily or which ended fatally.

A small number of lymphocytes would seem, on the whole to indicate better encapsulation, greater resistance, and a smoother convalescence after operation.

VACCINE THERAPY, A Study of Pathogen-Selective Cultures in Relation to, Boerner, F., and Solis-Cohen, M. Am. J. Clin. Path. 3: 125, 1933.

A series of 404 pathogen-selective cultures from 150 patients were studied.

In many cases the isolation of the more important pathogenic organisms was aided by the bactericidal action of the patient's fresh, whole, coagulable blood upon the less important and presumably nonpathogenic organisms.

Information was obtained regarding the presence or absence of bactericidal substances in the patient's blood for the various organisms isolated.

In about 11 per cent of the cases organisms were isolated which would have been missed by the ordinary methods of culturing.

A basis is provided for selecting the organisms to predominate in an autogenous vaccine, on the assumption that the organisms which grow in the patient's fresh, whole, coagulable blood are of most importance to the individual.

P. TULARENSE, A New and Simplified Medium for, Rhamy, B. W. Am. J. Clin. Path. 3: 121, 1933.

Formula of dehydrated "Bacto Cystine Heart Agar" (Difco)

Beef Heart, infusion from	500 gm.
Bacto peptone	10 gm.
Bacto dextrose	10 gm.
Sodium chloride	5 gm.
l-cystine	1 gm.
Bacto agar	15 gm.

Reaction: P_H 6.8. (The reaction may range between 6.8 and 7.3 but 6.8 gave the most luxuriant growth.)

Both the Bacto cystine heart agar and Bacto hemoglobin can be obtained in dehydrated form as a stock supply.

Method for preparing 500 c.c.

(A) "Double Strength Agar"—Dissolve, by boiling 28 grams Bacto cystine heart agar in 250 c.c. distilled water. Sterilize for twenty minutes at 15 pounds' pressure (250° F.). The sterile agar will be dark in color (chocolate agar).

(B) Dissolve 5 grams Bacto hemoglobin in 250 c.c. distilled water and strain through gauze to remove any large undissolved particles. Sterilize twenty minutes at 15 pounds' pressure.

(C) Cool both of the above sterile solutions (A and B) to 50° to 60° C. and mix. Dispense in sterile test tubes or other containers. Use strictly aseptic conditions. Incubate to test sterility.

AGGLUTININS, A Study of O and H Agglutinins in Typhoid and Endemic Typhus Fever, Kemp, H. A. Am. J. Clin Path. 3: 135, 1933.

Alcoholized E. typhi were used as O agglutinogen in agglutination tests carried out serially in six cases of typhoid fever. Positive agglutination was observed earlier than is usually observed in the Widal test.

Growth-phase selected O agglutinogen was positively agglutinated later than is usually found in the Widal reaction.

Formolized growth-phase O agglutinogen was agglutinated even later than untreated growth-phase selected agglutinogen and at much lower titers.

Both O and H agglutinins were found in the sera of typhoid-paratyphoid vaccinated individuals. The same individuals did not have O agglutinins prior to vaccination.

Both O and H agglutinins were found in the sera of individuals suffering with acute exacerbations in chronic arthritis.

Both O and H agglutinins for B. proteus X 19 were found in the serum of seven cases of endemic typhus (north Texas).

SEPTICEMIA, In the Newborn, Dunham, E. C. Am. J. Dis. Child. 45: 229, 1933.

Septicemia, in spite of the general impression to the contrary, is an important and relatively frequent cause of morbidity and mortality in the newborn. Blood cultures should

always be taken when a newborn infant becomes ill and the diagnosis is obscure. If the cause of the illness is determined early and transfusions of blood as well as other treatment are given, a number of these infants may recover.

Thirty-nine cases of septicemia in newborn infants occurring in a period of five years in the New Haven Hospital are reported. The final diagnosis, based on the growth of organisms from the blood, was usually corroborated by clinical, and, in many cases, by postmortem examination. The commonest organisms found were the streptococcus, the staphylococcus and B. coli.

Clinically septicemia due to the streptococcus seems to differ from other types of septicemia by the absence of jaundice and bleeding, by the less frequent enlargement of the spleen and by the more frequent appearance of cutaneous infections, omphalitis, peritonitis and meningitis. Streptococcus septicemia invariably had a fatal outcome.

In the cases of septicemia caused by the staphylococcus or by B. coli, on the other hand, jaundice was a common symptom, occurring in more than one-half of the cases in both groups. Bleeding took place in about one-half of the cases in the staphylococcus group and in about one-third of the cases in the B. coli group. The spleen was frequently enlarged (one-half or more of the cases) in both groups. Anemia, however, was less common in staphylococcus septicemia than in that caused by the streptococcus and B. coli. Infection of the urinary tract was found only in cases of B. coli septicemia. Staphylococcus or B. septicemia was not invariably fatal.

MALARIA, A New Method for Staining Thick Films, Chorine V. Bull. Soc. Path. Exot. **25**: 651, 1932.

Dry at a low temperature. Fix in 10 per cent commercial formalin until dehemoglobination is complete, three to fifteen minutes. Wash. Treat with a solution consisting of 3 c.c. of Lugol in 100 c.c. of a 2 per cent solution of potassium iodide, for three to five minutes. Wash. Stain in Giemsa, 2 drops to each 1 c.c. of hard tap-water, for one hour. The formalin dehemoglobinizes and fixes at the same time. It is advisable to add calcium carbonate to the formalin, to neutralize acidity. The optimum P_H is 7.3 to 7.5.

BILIRUBIN, SERUM: Relationship Between the Bilirubin Content of Serum From Different Types of Jaundice and the Icterus Index of the Serum After Removal of Proteins, White, F. D. Brit. J. Exper. Path. **14**: 17, 1933.

The residual icterus index is the name given to the icterus index when determined on serum from which the proteins have been removed by precipitation with alcohol.

It has been shown that in serum artificially rendered icteric by the addition of bilirubin, the residual icterus index is directly proportional to the bilirubin content in mg. per 100 c.c., the ratio being approximately 6 to 1.

Results have been obtained indicating that within the limits of this investigation, this relationship also holds for the serum of normal individuals and of jaundice cases of hemolytic origin, but does not hold for other types of jaundice.

The icteric ratio of serum is defined as the ratio of the icterus index to the residual icterus index.

In normal serum and icteric serum of hemolytic origin this icteric ratio has been found to be generally less than 1.2, whereas in all other jaundice cases investigated it was almost invariably much higher. Since this ratio is easily and quickly determined, it is suggested that in cases of latent or slight jaundice it might prove of assistance in differentiating such cases into "hemolytic" and "nonhemolytic" categories. A further differentiation of the latter category into "obstructive" and "toxic" types has not been found possible by this method.

It is claimed that the generally accepted normal values for serum bilirubin are too low, and that the normal range is at least 0.5 to 1.0 mg. and possibly 0.5 to 1.5 mg. per 100 c.c. serum.

PNEUMOCOCCUS, Studies of the Skin Reactions to the Specific Soluble Substances of the Pneumococcus Types I and II. Alston, J. M., and Lowdon, A. S. R. Brit. J. Exper. Path. **14:** 1, 1933.

In 281 persons who had not recently suffered from a pneumococcal infection, sensitiveness (increasing with age) to intracutaneous injection of pneumococcal S.S.S. type II was found in 63 per cent.

Reactors retested after five to twelve months were still sensitive in 92 per cent of cases.

Repeated tests at short intervals always produced a decrease, and in some cases a complete loss of sensitiveness.

In animals and human beings actively or passively immunized to pneumococci no immediate reactions could be produced by S.S.S. of homologous type. In rabbits, however, vaccination caused the development of a delayed form of reaction to the carbohydrate.

Skin sensitiveness (for immediate reactions) to the pneumococcal type-specific carbohydrates and circulating serum antibodies can each occur independently of the other and this work does not support the suggestion that the skin reations are associated solely with the recovery from pneumonia.

In the normal persons tested with intracutaneous injections of S.S.S. type II a considerable proportion showed as well as, or independent of, the immediate reaction, a delayed reaction. This was also found to be much more frequent in the older persons.

PELLAGRA, Erythrocytes In, Turner, R. H. Am. J. M. Sc. **185:** 381, 1933.

Using the most accurate available methods, erythrocyte counts and determinations of hemoglobin and percentages of packed red cells have been made for 70 bloods from 50 patients with typical pellagra.

Of the pellagrins 56 per cent showed no appreciable anemia; 16 per cent slight or questionable anemia; 12 per cent showed moderate anemia and 12 per cent a severe anemia, while 4 per cent showed an extremely severe anemia.

Two-thirds of the patients who died showed no anemia according to the methods and standards used.

Among those with severe anemia, other diseases which might have caused anemia were common.

Patients who suffered from diarrhea did not appear to be more anemic than those without diarrhea.

The influence of dehydration in obscuring anemia is discussed.

Anemia, when present, was definitely of the cholorotic, normocytic, or microcytic type, and in no instance of the macrocytic type.

Of the pellagrins 34 per cent had erythrocytes with corpuscular hemoglobin concentration less than normal, while for 66 per cent the concentration was within the normal range.

The average size of the red cells tended to diminish in proportion to the severity of the anemia, contrasting with the opposite rule for pernicious anemia.

The importance of the blood picture in the differential diagnosis between pellagra and pernicious anemia or sprue is emphasized.

LEUCOCYTES, Nonfilament Polymorphonuclear Neutrophil Count in Typhoid and Undulant Fever, Gallagher, J. R. Am. J. M. Sc. **185:** 391, 1933.

Total white blood cell counts and nonfilament polymorphonuclear neutrophil counts are recorded in 17 cases of typhoid fever and in 2 cases of undulant fever. These show a striking increase in the number of nonfilament, or immature, forms in the majority of instances.

The value of the nonfilament form count in the estimation of bone marrow response in these diseases is emphasized.

The suggestion is made that the increase in the nonfilament form of polymorphonuclear neutrophilic leucocyte is evidence that the leucopenia in these diseases is associated with white blood cell destruction rather than with bone marrow inhibition; but attention is

directed to the possibility that the increase in the number of young forms present may be a result of a bone marrow disturbance which inhibits the maturation of the neutrophils.

LEUKOCYTES, Filament-Nonfilament Count in Chronic Arthritis, Steinbracher, O., and Hartung, E. F. J. Am. M. Soc. **100:** 654, 1933.

The filament-nonfilament count is a useful routine diagnostic aid in chronic arthritis.

Filament-nonfilament counts in fifty patients with rheumatoid or chronic infectious arthritis were abnormally elevated in 100 per cent of the patients.

The filament-nonfilament count was normal in twenty-six patients, or 52 per cent, of a group of osteo-arthritic patients, while in the rest of this group the count was elevated.

The average nonfilament count was much higher (31.5 per cent) in patients with rheumatoid arthritis than in osteo-arthritic patients with an abnormal count (22.3 per cent).

The filament-nonfilament count is helpful in differentiating rheumatoid arthritis from osteo-arthritis only when within normal limits. A normal count indicates that chronic rheumatoid infection is not present. An elevated count may indicate the presence of rheumatoid arthritis, mixed rheumatoid and osteo-arthritis, or osteo-arthritis with active focal infection.

TUBERCLE BACILLI: Comparison of Certain Media for Cultivation of From Sputum, Shaffer, M. F. Am. Rev. Tuberc. **27:** 259, 1933.

In this study 6 media have been compared as to their relative value for the cultivation of the tubercle bacillus from sputum, following preliminary treatment of the 42 specimens with 6 per cent sulphuric acid according to the slightly modified Corper-Uyei method.

The medium of Corper and Uyei yielded the largest number of positives, being equal to the microscopic method in total number of results. Because of simplicity of preparation and the excellent results obtained through its use, this medium is particularly recommended for primary cultures of the tubercle bacillus following acid-pre-treatment.

The medium of Petragnani is likewise highly recommended, since it yielded nearly as good results as those obtained with the Corper-Uyei medium. These two were superior to the other four media tried in numbers of positives, at all times.

Lubenau's medium proved slightly superior to the media of Dorset, Petroff and Sweany-Evanoff, all of which gave practically the same results.

In this series the culture method has proved itself to be at least the equal of microscopic examination and, if anything, slightly superior to it. Out of 42 specimens examined, the microscopic method showed 33 positives while the culture tubes revealed 37 positives.

In the routine isolation of tubercle bacilli from sputa, it is recommended that both the medium of Corper-Uyei and that of Petragnani be employed.

Dissociation of the primary cultures into colonies resembling the so-called R and S types has been noted particularly on Petroff's medium.

TISSUE: Rapid Method for, Erskine, E. B. J. A. M. A. **100:** 573, 1933.

Fresh tissue is cut into convenient size and about 2 mm. thick. This tissue is placed in a test tube of neutral "solution of formaldehyde U. S. P.," diluted 1:10 and gently boiled for a period of three minutes, during which time five test tubes, appropriately labeled and containing respectively (1) 80 per cent alcohol, (2) 95 per cent alcohol, (3) 100 per cent alcohol, (4) chloroform and (5) 95 per cent alcohol are placed in a water-bath and the temperature brought to 55° C.

Temperature is accurately gaged by a thermometer placed in the test tube prior to placing the tissue in the tube, whereupon the thermometer is dried and placed in the succeeding tube solution. It will be noted later that Tube 5, containing 95 per cent alcohol, serves no purpose other than a check on the temperature during the time the tissue is in Tube 4.

The fixation of the tissue in boiling formaldehyde solution having been completed, the

solution is poured off and the tissue dried on blotting paper. It is then placed successively in the solution contained in Tubes 1, 2, 3 and 4, for a period of five minutes in each.

The next two steps are carried out in the embedding oven at the same temperature (55° C.). Chloroform and paraffin (equal parts) and paraffin are always kept in the oven at the temperature mentioned and consequently are always available for this method. The tissue is kept in each solution for five minutes, whereupon it is embedded in paraffin, immediately chilled in a pan of ice water, cut and mounted on a slide.

ANEMIA, Primary Hypochromic, Dameshek, W. J. A. M. A. **100:** 540, 1933.

The clinical features of twenty-five cases of primary hypochromic anemia were studied.

The disease is characterized by the presence in adult women of a chronic idiopathic "secondary" anemia, subject to remissions and relapses.

It presents the following clinical features:

Symptoms.—Weakness, dyspnea, gastrointestinal complaints, sore tongue, paresthesias, weight loss.

Signs.—Pallor without icterus; wrinkled, atrophied, inelastic skin, glossitis, brittle, often spooned, finger nails, dry, gray hair.

Blood.—Anemia of low color index; hypochromia of the red blood cells, small average red blood cell diameter, leucopenia, and reduction in blood platelets.

Gastric Juice.—Achlorhydria, usually complete, small volumes, increased mucus.

Response to Medication.—Prompt and striking response to large doses of inorganic iron; small doses of copper sulphate of benefit when the anemia becomes "fixed."

Certain cases are associated with pregnancy, dysphagia (Plummer-Vinson syndrome), gastrectomy and gastroenterostomy, myxedema, and tapeworm infestation. The common feature is achlorhydria.

On the basis of striking response to treatment with inorganic iron, there is a possibility than an iron deficiency is present. It is possible that absence of free hydrochloric acid from the gastric juice may result in poor absorption of food iron from the gastrointestinal tract.

Anemia of Pregnancy, Rowland, V. C. J. A. M. A. **100:** 537, 1933.

Liver extract is specific in pernicious or hypochromic anemia of pregnancy. The anemia is due to a relative or temporary deficiency of a specific hemogenic substance produced in normal gastric digestion. This substance is apparently identical with that lacking in true Addisonian anemia.

Iron in dosages of from 90 to 120 grains a day is specific in the secondary or hypochromic anemia of pregnancy. This anemia is probably due to dietetic deficiency and to faulty absorption of the hematogenic substance on account of gastrointestinal disturbances.

These anemias occur in slight degree in the majority of American women during pregnancy and are associated with hypochlorhydria and a deficiency in the specific intrinsic factor involved in blood formation.

The mechanism of hematogenesis, ordinarily quite adequate, is apparently overstrained by the metabolic overload of pregnancy and likewise by various infections, toxic agents, and marked nutritional deficiencies.

Pernicious anemia can hardly be regarded as one specific disease but rather as a nonspecific failure of hematogenesis, due to a variety of causes that operate through the common mechanism of a deficiency or disorder of gastric function.

In the rare case of severe pernicious anemia of pregnancy, the intramuscular or intravenous use of liver extract may be life saving either before or after delivery. The response may be noted within thirty-six hours. If the patient survives the first five days, she may be expected to recover.

Transfusion is useful as an emergency measure only.

Termination of pregnancy is indicated mainly by serious complications.

Obstetric morbidity and mortality statistics might be improved by applying the newer methods of treatment and prophylaxis in anemia in prenatal work in order to bring the blood up to full normal.

TRANSFUSION, Prevention of Chills Following Transfusion of Citrated Blood, Lewisohn, R., and Rosenthal, N. J. A. M. A. **100**: 466, 1933.

The authors emphasize the necessity for a centralized department for the thorough cleansing and preparation of transfusion apparatus for the citrate method, report the practical elimination of chills when this is done, and describe their method as follows:

1. *Distilled Water, Sodium Citrate and Sodium Chloride.*—Triple distilled water, obtained from a special Barnstead still, is used for the preparation of sodium citrate (30 per cent) and sodium chloride (0.85 per cent).

Sodium citrate (30 per cent) may also be bought on the open market in 5 c.c. ampules. One should inquire from the manufacturer for sodium citrate prepared with triple distilled water. For actual use, 1 c.c. of this concentrated sodium citrate solution is used for every hundred cubic centimeters of blood.

2. *Special Cleansing of Apparatus.*—After each transfusion, all parts are separated and washed in cold water for the removal of blood. They are then washed in a dilute solution of green soap to which compound solution of cresol has been added to make up about a 1 per cent solution. They are then thoroughly rinsed in tap water.

All parts are then placed in a large pan containing sodium hydroxide (0.1 per cent solution) and boiled for five minutes. They are then transferred to a large pan containing distilled water, to remove the sodium hydroxide. The glassware and rubber tubing are again washed with triple distilled water and are ready to be assembled and sterilized, either in metal boxes or in special bundles in the autoclave.

The glassware and rubber tubing are boiled separately. The needles are always sharpened before being treated but boiled for only three minutes in sodium hydroxide solution (0.1 per cent).

3. *Preparation of Apparatus for the Autoclave.*—A. For the donor: A bundle containing the apparatus for taking the blood from the donor (two pyrex cylinders 500 and 10 c.c., respectively, one glass rod, two tourniquets, two needles gage 13 and 15, Luer adapter and rubber tubing, one ampule of 30 per cent sodium citrate).

B. For the recipient: A metal box, containing glassware, rubber tubing and cannulas for the transfusion into the recipient.

Aschheim-Zondek Test, Modification, Rudd, G. V., and Ingram, W. W. Med. J. Australia **2**: 629, 1932.

The following modification enables the test to be read in 55 to 72 hours.

The early morning specimen of urine, slightly acid to litmus, is mixed with three volumes of absolute alcohol and allowed to stand for thirty minutes. The mixture is centrifugated and the supernatant fluid is poured off. The precipitate is taken up in a small volume of water and the mixture is again centrifugated. The aqueous extract is found to be highly active. The precipitate may be spun down in a 100 c.c. centrifuge tube in three spins, the supernatant fluid being discarded each time. The supernatant fluid is decanted off and the tube drained for a few minutes, or, if the precipitate is bulky, the tube is placed in a water-bath at 40° C. and exhausted for a few minutes in order to remove most of the alcohol. The precipitate is taken up with 1.5 c.c. of distilled water and transferred to a 10 c.c. centrifuge tube. The large centrifuge tube is washed out with three quantities of 0.7 c.c. of distilled water and the washings added to the 10 c.c. centrifuged tube. After thorough mixing, the insoluble portion is centrifuged out. The concentration effected by the procedure is about 15 to 1. After preliminary tests with the concentrates prepared in this manner, the preparation of the concentrate from urine was commenced in the morning. Four mice, from twenty-eight to thirty days old, receive two injections of from 0.3 to 0.4 c.c. at an interval of six hours. Fifty-five hours after the first injection, the ovaries of two of the mice were examined. If hemorrhagic follicles were not seen, the remaining mice were examined about seventy hours after the first injection. The finding of one hemorrhagic follicle constitutes a positive reaction. In the absence of hemorrhagic follicles, the result of the test is "negative."

REVIEWS

Books and Monographs for Review should be sent direct to the Editor, Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

Specific Changes in the Blood Serum*

BENDIEN will be recognized as the originator of a recently described serologic test for malignancy based upon a disturbance of serum lability in the presence of a malignant process and elicited by flocculation of the serum in the presence of varying concentrations of orthosodium vanadate and tenth normal acetic acid.

In this volume is presented a short account of the researches upon which the test is based. The book is not intended as a manual for the laboratory worker and presents the subject in broad outline rather than in detail.

While the results reported by Bendien have not as yet been corroborated by other workers, the work presented represents extensive and serious scientific study and as such deserves the consideration of all concerned with the subject.

The volume should be of interest for its contribution to the study of malignancy and as such demands the attention of workers in this field.

The Microbiology of Foods†

THIS is a book which undoubtedly will take a foremost place on the working shelf of the laboratory worker and will soon acquire the honorable scars of frequent use.

Microbiology as concerned with the desirable and undesirable activities of bacteria in their relation to foodstuffs is a branch of bacteriology which has undergone extensive expansion within recent times.

Much of the information, practical as well as theoretical, which has accumulated as a result of the numerous and varied studies in this field have not been readily accessible either to the laboratory worker or the physician in general. Now, in this book, it has been gathered, reviewed, summarized, and presented in an orderly, systematic, and comprehensive manner.

In its presentation of the subject this volume is eminently practical.

Whether one wants to know what studies have been made of foodstuffs or the manner in which they may be acceptably prepared commercially; or what constitutes a satisfactory standard; or how to determine if a particular sample conforms to this standard; all will be found clearly outlined and discussed.

The subjects of food poisoning and food infection, while of course referred to are not discussed in detail, this being reserved for a separate volume.

As said above, the book may well take its place as a standard reference to be read with profit by the physician at large as well as the laboratory worker.

Primary Syphilis in the Female**

THIS volume is based upon the experience of the author as Supervisor of the Venereal Clinic at St. Thomas' Hospital, London, since 1917.

Primary syphilis in the female is not only rarely seen by the average practitioner but has not been subject to any extended or detailed account in the literature. This volume, therefore,

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*Specific Changes in the Blood Serum. By S. G. T. Bendien, translated by A. Piney, M.D. Cloth, 95 pages, 64 illustrations. The C. V. Mosby Co., St. Louis, Mo.

†The Microbiology of Foods. By F. W. Tanner, Professor of Bacteriology, University of Illinois. Cloth 768 pages. The Twin City Printing Co., Champaign, Ill.

**Primary Syphilis in the Female. By Thomas Anwyl Davies, Cloth, 111 pages, 24 figures and 12 colored plates. Oxford University Press.

fills a needed gap and does so in an excellent and comprehensive manner. Attention is called to the fact that of 584 primary genital chancres in the female, 45 per cent were internally situated, 44 per cent being on the cervix which, therefore, should be examined as a routine.

The typography, arrangement of the material, and the illustrations are excellent, the whole making a very valuable book worthy of a place in the library of every physician.

Laboratory Service and the General Practitioner*

THIS book is not intended to be, nor is it, a manual of laboratory technic, but has for its purpose the interpretation and application of laboratory work in clinical purpose.

Books of this kind are sorely needed and the present volume, though small, should be welcomed by the physician for the information it gives of how and when to utilize the methods of the laboratory and how to interpret in terms of clinical applicability to the patient, the reports rendered.

An appendix contains an alphabetical list of disease and the most informative laboratory examinations applicable.

The book deserves a warm reception by the practitioner.

Behavior Aspects of Child Conduct†

BEHAVIOR is a word implying genetic interest and conduct a word implying a moral and philosophic interest in a person's actions.

The views of former generations that annoying behavior in a child was due solely to "badness" or nervousness have largely been replaced by those regarding behavior as an aspect of mental or physical health.

This concept has been so confused by a multitude of psychological and psychiatric theories and so buried in a bewildering mass of words that this practical and understandable volume by Dr. Richards will be received with sincere gratitude by its readers.

The book embodies a series of lectures delivered under the auspices of the Child Association of America and designed, not so much to present or enter into theoretical discussions, as to present some help in the solution of individual behavior problems.

This purpose is well and satisfactorily achieved and the book can be recommended for lay as well as professional readers.

Public Health in New York State**

IN 1930 Governor Franklin D. Roosevelt of New York appointed a Special Health Commission to survey and report upon the administrative and legislative aspects of public health and to make whatever recommendations it might deem advisable to lessen, by means of better utilization of existing knowledge, the gap between what is achieved and what might be realized in the conservation of public health.

In this volume is presented the detailed and comprehensive report of the commission.

It may well be read by all concerned with the problems of public health for its wide vision, careful and thorough presentation, and the value of the recommendations made.

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*Laboratory Service and the General Practitioner. By Arnold Renshaw, Director, Laboratory of Applied Pathology and Preventive Medicine, Manchester, etc. Cloth, 267 pages, 7 figures. Oxford University Press.

†Behavior Aspects of Child Conduct. By Esther L. Richards, Associate Professor of Psychiatry, Johns Hopkins School of Medicine. Cloth, 299 pages. The Macmillan Co., New York.

**Public Health in New York State. Cloth, 504 pages, 37 charts, numerous photographs. New York State Department of Health, Albany.

The example set by the appointment and work of this commission could be very profitably followed by the country at large.

Diagnosis and Treatment of Venereal Disease in General Practice*

THE fourth edition of this well known book has been thoroughly revised and will doubtless receive the warm reception deserved and accorded to its predecessors.

Introduction to Practical Bacteriology†

THIS is the third, thoroughly revised edition of a manual designed primarily for students of the University of Edinburgh, but which even the experienced laboratory worker may read with profit.

It is neither an epitome of bacteriology nor an abbreviated textbook but an eminently practical introduction and guide to bacteriology which can be well recommended as excellently fulfilling the purpose in view.

Quarterly Bulletin of the Health Organization, League of Nations**

THE purpose of this publication, while not an official organ, is to render available and more accessible in English and French, the reports and records of the organization.

This first number contains the following:

1. Immunization Against Diphtheria: Resolutions of the Conference Held in London, June, 1931.
2. Medical Education in England.
3. The Milk Supply of North-American Cities.
4. Tropical Pneumonia.
5. Second Analytical Review of Reports from Pasteur Institutes on the Results of Anti-Rabic Treatment.
6. The Floods in China.

From this table of contents it is evident that the volume appeals to varied interests.

The report of the comprehensive study of diphtheria immunization embodies the results of an investigation begun in 1928 and carried out in a number of countries.

Its recommendations are of sufficient importance to quote in full.

Immunization against diphtheria, when carried out under suitable conditions and with efficient prophylactics, effects a large reduction in the diphtheria mortality and morbidity rates among children thus treated. This is proved by studies carried out under strictly comparable conditions, such as those observed during the recent inquiries.

The reactions sometimes observed after the injection of diphtheria prophylactics give no cause for alarm and should not interfere with the campaign for the immunization of children, including tuberculous children, nor should they prevent the choice of the most active prophylactics.

The conclusion appears justified that, in spite of a few exceptional cases, the efficacy of immunization may be deduced from the change of the Schick reaction from positive to negative.

Taking as a basis the figures concerning the morbidity, the percentage of Schick reactions rendered negative after the injections of the immunizing agent, and a certain number of titrations

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*Diagnosis and Treatment of Venereal Diseases in General Practice. By L. W. Harrison, Director, Venereal Department, St. Thomas' Hospital, with a chapter on The Medico-Legal Aspects, etc., by F. G. Crookshank. Cloth, 567 pages, 29 figures, Fourth edition. Oxford University Press.

†Introduction to Practical Bacteriology. By T. J. Mackie, Professor of Bacteriology, University of Edinburgh, and J. E. McCartney, Director of Research late Metropolitan Asylums Board, London. Cloth, 421 pages, Third edition. Oxford University Press.

**Quarterly Bulletin of the Health Organization, League of Nations. Vol. 1, No. 1, Paper, 157 pages. Obtainable from World Peace Foundation, Boston, Mass.

of antitoxin in the blood of persons immunized, it may be concluded that formol toxoid (anatoxin) is the most efficient of those antigens which have been the subject of our comparative study, i. e., mixtures of toxin, antitoxin and formol toxoid (anatoxin), and should be recommended for the present.

The clinical observations submitted to the conference indicate that, so far as can be determined up to the present, the immunizing power of formol toxoid (anatoxin) in man appears to be in relation with the antigenic value of the prophylactic as measured by the flocculation method.

The method of administration recommended is by subcutaneous injection. When this is not possible, the prophylactic may be applied to the nasal mucous membrane. Our experience with the cutaneous method has not been favorable.

Immunization should be carried out by means of three injections. It is hoped that, in the future, the use of a more active prophylactic will make it possible to immunize with two or perhaps even one injection.

The intervals recommended between injections are three weeks between the first and second injections, and at least two weeks between the second and third.

It is unnecessary to carry out the Schick reaction before immunization. It is, however, desirable to use the Schick reaction before and after immunization in a certain proportion of subjects in order to determine the value of the method employed.

It is recommended that immunization against diphtheria should be carried out not later than during the pre-school period, after the end of the first year of life.

If the children have not been immunized during the pre-school period, they should be immunized, if possible, during the first year of school attendance.

Charitable organizations and administrations receiving children in homes (holiday camps, "preventoria," sanatoria, etc.) are advised to require, from both children and staff, a diphtheria immunization certificate on admission or a certificate attesting that the Schick reaction was negative.

Immunization is recommended for medical, nursing and domestic staff in hospitals, homes, dispensaries, sanatoria, schools, etc.

Immunization is to be recommended even during epidemics in the case of children who have been in contact with patients, as there is so far no evidence on record as to the existence of a negative phase.

In the opinion of the experts, diphtheria immunization should form the subject of active public education on the part of the health administrations of the different countries in order to bring home to everyone the advantages of this method of protection in safeguarding the public health.

The report and study of Medical Education in England by Sir George Newman, Chief Medical Officer of the Ministry of Health and of the Board of Medical Education, England, is of extraordinary interest and value. It is regrettable, indeed, that this study cannot be made generally available for it should be read, not only by all associated with medical education, but by all physicians.

The views expressed are based upon an extensive practical experience and deserving of the highest consideration, focussing upon the belief that "the objective is a good, all-around practical medical man, capable of thinking for himself, resourceful and competent to deal with the principal needs of his patient."

That the present scheme of medical education too often falls short of this objective is no secret and the remedies proposed by this author well deserve wide circulation and thoughtful consideration.

The report on Milk Supply by Professor R. Burri concerns an investigation of this problem conducted in the United States, while that on Tropical Pneumonia is comprehensive and detailed and emphasizes the difference in symptomatology, together with a survey of methods for its treatment and prophylaxis.

The report of the Pasteur Institute is largely statistical but contains much of interest; while the report on the floods in China describes the campaign instituted against epidemics.

It may well be said that seldom is so much information of interest and value available for the price of this pamphlet.

The Diet Book*

THE proper feeding of the sick, especially when this has to be done at home, is often a problem of no minor degree, for not only are invalids notoriously capricious as to appetite, but special and carefully balanced menus are often desirable if not essential.

The purpose of this book, as set forth in the preface, is: "To simplify for the general practitioner the labor of detailing to his patients what they may or may not eat; to indicate to the patient himself a dietary suitable, not too monotonous, but at the same time palatable; and to give to the harassed housewife practical suggestions . . . . while keeping within the bounds of a prescribed diet."

These purposes are excellently achieved in a concise and practical manner. Special diets are set forth for a week at a time in an easily understandable way and a wealth of recipes and practical information is presented in an attractive manner.

The book is well conceived and the plan well carried out. It should be most useful to all concerned.

Breast Feeding†

PERHAPS the common tendency of modern times to wean babies early and avoid, for a variety of reasons, the "strain" of breast feeding, would be less marked if this eminently practical and well-written book could be read by every physician and every expectant mother.

It can be recommended without reserve as an excellent exposition of a highly important subject.

An Introduction to Pharmacology and Therapeutics**

THIS small volume is addressed primarily to the student and intended to provide a short and comprehensive survey of the subject to supplement and not replace lectures.

Well written, practical, and concise it well serves the purpose in view.

The Individuality of the Blood‡

THIS book constitutes an invaluable contribution to the literature of a most important subject and will, without doubt, be the standard reference text for years to come.

The term "individuality of the blood" signifies those constitutional peculiarities of the blood which enable a distinction between individuals of the same species, a discussion of which, in a strict sense, forms the subject matter of this volume.

In conformity with this definition, although much space is naturally given to a discussion of blood groups and their corollaries, the author has not restricted himself to this field but discusses many other phases of the individuality of the blood as related both to clinical and forensic medicine.

The present volume is an excellent translation of the French edition of 1929 which has been extensively revised by Professor Lattes and brought into conformity with the latest advances in this field.

Following an introductory chapter the subject is discussed under the following subdivisions: Individual Reactions of Normal Blood; The Hereditability of the Individuality of the Blood;

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*The Diet Book, for Doctor, Patient and Housewife. By Marguerite R. Rea. Cloth, 197 pages. Oxford University Press.

†Breast Feeding. By Margaret Emslie, Late Senior Assistant Medical Officer Maternity and Child Welfare, County Borough of Croydon, etc. Cloth, 142 pages, 8 plates. Oxford University Press.

**An Introduction to Pharmacology and Therapeutics. By J. A. Gunn, Professor of Pharmacology, University of Oxford. Cloth, 220 pages. Oxford University Press.

‡The Individuality of the Blood, in Biology and in Clinical and Forensic Medicine. By Leone Lattes, Director of The Institute of Forensic Medicine, University of Modena, Translated by L. W. H. Bertie. Cloth, 413 pages, 71 illustrations. Oxford University Press.

The Individuality of the Blood as an Ethnoanthropological Fact; The Individuality of the Blood in Forensic Medicine; and, finally, The Technic of the Individuality Reactions.

The section on the hereditability of the blood groups has assumed within recent years a definite medico-legal importance in questions of parentage and legitimacy and is discussed in extenso, leading to the following conclusions:

"1. The hereditary transmission of the blood group is an established fact.

"2. This transmission takes place according to Mendel's law and the isoagglutinable properties A and B behave as Mendelian dominants.

"3. We may take it as definitely established that the transmission of the blood groups is effected by means of two allelomorphic characters, one being derived from the father and the other from the mother; the possible allelomorphs are three in number (multiple allelomorphs), their combination in pairs giving rise to six genotypical blood groups in man."

The chapter on forsenic applications may be taken as the last word to date upon this subject.

As complicated as is this subject, the author's handling of it is remarkably clear, understandable and easily read, a tribute to the skill of the translator. A bibliography, comprising only significant contributions utilized in the text covering 91 pages, testifies to the thoroughness with which the subject has been reviewed.

This book may well be considered an epoch-making contribution of the highest importance and deserves the widest possible circulation.

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EDITORIAL

Focal Infection and the Tonsils

THE acceptance of the tonsils as foci of infection connected with the production of systemic disease has been so extensively studied and so commonly assumed as to render it questionable if, at times, the discovery of infected tonsils has not been made the focus rather than an incident of physical examination.

Infected tonsils may, beyond doubt, be a primary cause of widely disseminated lesions, but it is also not unlikely that this may be a post hoc propter hoc assumption, and it is, therefore, of some interest to survey observations pertinent to the question.

Such a survey, for example, is that of Pavlogt and Crowe[1] who reviewed the bacteriology of the tonsils and adenoids in 100 selected cases, 50 per cent being children under eleven years of age.

Their purpose was to determine: (1) The predominating organism deep in the crypts; (2) whether there was any difference between the organisms re-

covered from cases with systemic disease and those in which only local symptoms occurred; and (3) to determine the presence or absence of a carrier state.

A conclusion of practical importance was that a surface culture secured by swabbing the tonsil was a reliable index of the predominating bacteriology of the crypts.

Streptococcus hemolyticus was recovered in 91 cases, 50 per cent being children, whereas the majority of those showing staphylococci as the predominant organism were over twenty-five years old.

In 81 per cent both tonsils and adenoids harbored hemolytic streptococci while in ten cases streptococci were found in the tonsils, and staphylococci in the adenoids.

In a somewhat similar series[2] of 409 unselected cases, in the majority of which the tonsillar infection was of some standing, the results were as follows:

Streptococci, 6.4 per cent; Staphylococcus aureus, 33 per cent; M. catarrhalis, 34 per cent, and pneumococci (all types), 54 per cent.

These and numerous similar observations suffice to establish the tonsils as potential factors of importance in the production of systemic disease and have led, very naturally to attempts to establish a clear-cut correlation of tonsillar infections with specific diseases.

Kaiser[3] from a study of the relation of the tonsils to acute rheumatic fever in childhood involving 439 cases, draws the following conclusions:

1. The most susceptible age for the first attack is between eight and fourteen years, the first attack most often developing while the tonsils were still present.

2. The incidence of recurring attacks was 10 per cent less when the tonsils were removed than when they were not removed.

3. Carditis as a complication was apparently not influenced by tonsillectomy, as was true also of chorea. On the other hand, the association of carditis and chorea was less frequent after tonsillectomy.

In view of these findings Kaiser believes that the tonsils are avenues of infection in many cases of rheumatism and that, until more is known of the etiology of this disease, their removal is indicated in the rheumatic and potentially rheumatic child.

Lest this should serve as a warrant for promiscuous and indiscriminate tonsillectomy, the same observer[4] from a careful analytical study of twenty-two hundred tonsillectomized children, with an equal number of controls, three and ten years after operation, concludes:

1. That the real value of removal of tonsils and adenoids cannot be definitely established in a few years, as many apparent early postoperative benefits are not equally evident over a ten-year period.

2. That one of the outstanding benefits over a ten-year period is the influence upon the incidence of "sore throats" which show an approximate 25 per cent decrease.

3. That the apparent decrease in the incidence of "head colds" and otitis media evident during the three-year postoperative period was not maintained over the ten-year period; while, strangely enough, respiratory infections, such as laryngitis, bronchitis, and pneumonia, were more frequent in the tonsillectomized group.

4. That while not striking, there was a definitely perceptible difference in the susceptibility of the tonsillectomized and nontonsillectomized groups to diphtheria and scarlet fever, the difference being in favor of those in whom tonsillectomy had been done.

5. That during the ten-year period there was a marked reduction in the incidence of cervical adenitis.

6. That in the tonsillectomized group, and especially when tonsillectomy was done early, first attacks of rheumatism were from 30 to 50 per cent less frequent. Where recurring attacks were present no benefit was seen following tonsillectomy.

In view of these findings there can be no dissent upon the dicta laid down by Kaiser that tonsillectomy should be regarded as a major operation; that incomplete removal by no means exhibits the same aftermath as complete extirpation in relation to the incidence or prevention of disease; and that the hazards of tonsillectomy must always be considered in the evaluation of the end-results.

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R. A. K.

NEWS AND NOTES

The Maryland Society of Registered Technicians

The Maryland Society of Registered Technicians held their annual meeting in the auxiliary parlors of the Mercy Hospital, Baltimore, Maryland, on April 29, 1933. The following officers were elected: Sister M. J. Wilson, President; V. L. Flannery, Secretary and Treasurer. E. P. Walker and N. E. Behr were appointed members of the board of directors.

This society, which consists of registered laboratory technicians, will meet in the future on the first Saturday of the months of September, November, January, March, and May.